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Review

Pharmacokinetics and metabolism of nicotine

Piotr Tutka[1], Jerzy Mosiewicz[2], Marian Wielosz[1]

[1]Department of Pharmacology and Toxicology, Medical University of Lublin, Jaczewskiego 8, PL 20-090 Lublin, Poland

[2]Department of Internal Diseases, Medical University of Lublin, Staszica 16, PL 20-081 Lublin, Poland

Correspondence: Piotr Tutka, e-mail: tutkap@itsa.ucsf.edu or tutka@am.lublin.pl

Abstract:
Nicotine (NIC), the major constituent of tobacco, is responsible for the compulsive use of tobacco. Advances in understanding of the pharmacokinetics and metabolism of NIC have been made rapidly over the past decade. The application of highly sensitive gas chromatography/mass spectrometry led to the identification and quantitation of new NIC metabolites as well as characterization of new pathways of NIC biotransformation. This review summarizes findings from human and animal studies concerning NIC kinetics and biotransformation as well as describes the factors that influence these processes.
Recently, large individual, racial and species differences in the metabolism of NIC have been well documented. The differences in the metabolism of NIC may be a result of genetic, environmental, and developmental host influences. We review the scientific evidence from studies that supports a role for genetic mechanisms responsible for variability in the profile and the rate of the NIC metabolism. Actually, the majority of the genetic studies focus on the characterization of the CYP2A6 gene polymorphism, and on determining the relationship between the phenotype of NIC metabolism and the genotype of the CYP2A6 gene. There is good evidence that genetic polymorphisms associated with NIC metabolism are an important factor responsible for susceptibility to NIC dependence. It is anticipated that genetic findings can lead to the identification of individuals at a greater risk for tobacco addiction and will be used for more effective treatment and prevention strategies to reduce smoking.

Key words:
nicotine, cotinine, tobacco, smoking, nicotine metabolism, CYP2A6

Abbreviations: COT – cotinine, NIC – nicotine

Introduction

Cigarette addiction, the most common form of tobacco product addiction, continues to be one of the world's most serious public health problems. Cigarette smoking is considered to be the major risk factor of ischemic heart disease [53] and is strongly linked to lung cancer and chronic obstructive pulmonary disease [25]. Besides its direct effect on health, smoking influences the pharmacokinetics and pharmacodynamics of many drugs and can be responsible for ineffectiveness of medical therapy, or drug toxicity.

The widespread use of cigarettes is caused by addiction to nicotine (NIC). NIC is a tertiary amine composed of a pyridine and a pyrrolidine ring (Fig. 1). It is well known that NIC exerts a number of cardiovascular and behavioral effects. The actions of NIC are initiated by binding to nicotinic cholinergic receptors in the autonomic ganglia, adrenal medulla, neuromuscular junctions as well as in the brain and spinal cord, resulting in the release of a number of vasoactive catecholamines and neuroactive peptides that mediate sensitivity and tolerance to NIC [47, 48]. Recent

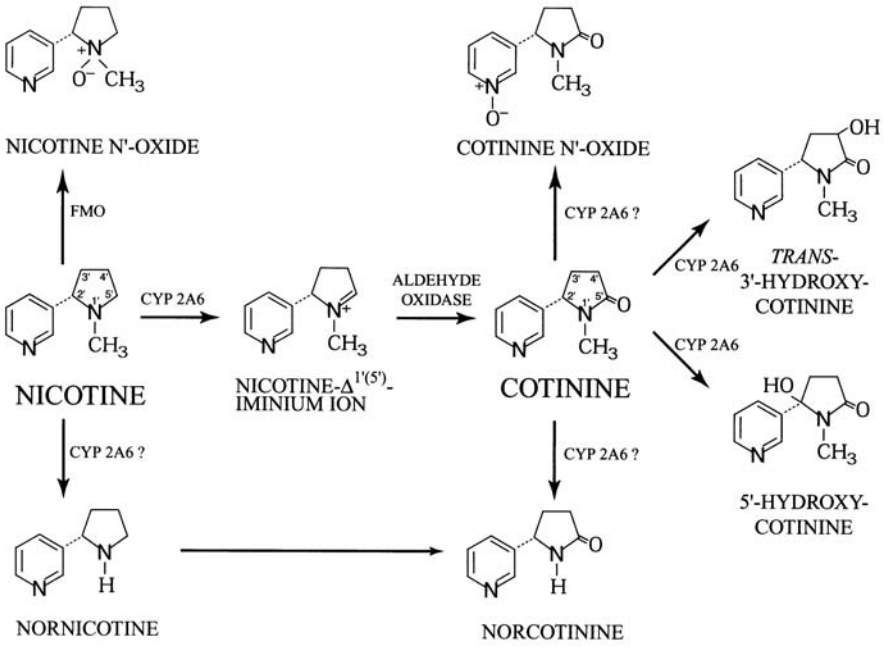

Fig. 1. The majors pathways of nicotine metabolism in humans

findings suggest that alterations in nicotinic receptors may lead to neurological diseases, some associated with increased incidence of smoking [47].

Pharmaceutical NIC available as chewing gum, transdermal patches, nasal spray, inhalator or microtablets is used in the replacement therapy for stopping smoking. NIC is also being investigated as a therapeutic agent in the treatment of Alzheimer's disease, neuropsychiatric motor disorders, and ulcerative colitis [1].

Characterization of NIC kinetics and metabolism is very helpful in our understanding of its involvement in the pathogenesis of smoking-related diseases. Moreover, advances in understanding of NIC pharmacology are necessary for optimization of NIC replacement therapy and the development of medications for treating tobacco addiction. Furthermore, an improved understanding of NIC pharmacology may clarify some aspects of smoking behavior.

Kinetics of nicotine

Nicotine is absorbed from cigarettes through the lungs. Pulmonary absorption of NIC is extremely rapid, occurring at a rate similar to that after intravenous administration [74]. Absorption of NIC is pH-dependent [80]. At an acidic pH of smoke from cigarettes (pH 5.5) NIC is mostly ionized and does not readily permeate cell membranes [80, 93]. Therefore, in the lungs NIC is buffered to a physiological pH and rapidly crosses membranes.

NIC from smokeless tobacco (snuff and chewing tobacco) is absorbed through the oral mucosa. At an alkaline pH of smoke from tobacco in pipes, NIC is mostly non-ionized and well absorbed from the mouth [86, 93]. Similarly to cigarettes, there is a considerable variation among individuals in the amount of NIC absorbed from smokeless tobacco, even when they all place the same-sized dose in their mouths [5]. The levels of NIC in the plasma rise over 30 min and slowly decline over the next hours [14]. The regular use of smokeless tobacco results in the plasma concentration of NIC comparable to those seen in cigarette smokers [14].

One of the most effective pharmacological adjuncts to smoking cessation therapy is NIC substitution by use of NIC gum or transdermal patch, which can relieve or prevent withdrawal symptoms and facilitate tobacco abstinence [38]. Most NIC replacement therapy forms deliver NIC more slowly than smoking.

Tab. 1. Nicotine and cotinine pharmacokinetics in smokers and non-smokers

	Nicotine		Cotinine	
	S	NS	S	NS
Half-life (min)	157 ± 78	122 ± 45	1047 ± 304	1012 ± 259
Volume of distribution (l)	196 ± 74	185 ± 63	54 ± 16	58 ± 12
Total clearance (ml/min)	1085 ± 282	1319 ± 567	40.6 ± 11.1	45.1 ± 15.7

From Benowitz [4]. S – smokers; NS – nonsmokers. Table data are expressed as means ± SD

Absorption of NIC from the gum is gradual and the total amount of absorbed NIC is significantly lower compared to the amount of NIC contained in the gum. The plasma levels of NIC after chewing the gum are lower than the levels after cigarette smoking [14]. Frequent dosing is necessary to achieve good NIC absorption from the oral mucosa. Also, correct chewing technique is required to obtain adequate plasma concentration of NIC.

Transdermal NIC patch, the most comfortable form of NIC replacement therapy, is administered once daily. NIC from the patch is slowly absorbed; its plasma concentration rises gradually over 6–10 h and tends to reach a plateau over the next 8–12 h (depending on the type of patch), declining slowly over the final 6 h [3, 81]. Like NIC gum, the patch does not allow to achieve the plasma NIC levels of heavy smokers. Furthermore, consideration has to be given to the particular factors influencing cutaneous blood flow. Vasoconstriction or vasodilatation due to the changes in skin temperature, nervous factors or vasoactive drugs can affect the NIC absorption from transdermal patch.

NIC nasal spray is a new and less common method of NIC replacement therapy. NIC delivered from nasal spray is absorbed through the nasal mucosa. The absorption is very rapid and the peak arterial plasma levels are reached in about 5 min after administration [93]. There is approximately a fivefold individual variability in the absorbed dose of NIC and its plasma levels during the nasal spray use [15].

Following absorption, NIC readily reaches many organs and tissues and undergoes extensive metabolism. About 10–20 s after absorption NIC is present in the brain [2, 65]. Penetration of NIC across the blood-brain barrier occurs by both passive diffusion and active transport by the choroids plexus [79]. Rapid NIC uptake into tissues and intensive metabolism lead to its quick disappearance from the plasma. Since NIC is eliminated from the body very rapidly, considerable fluctuations in the NIC plasma concentrations can occur during cigarette smoking.

A summary of the NIC pharmacokinetics in humans is shown in Table 1. The elimination half-life of NIC averages 2 h with significant variability from 1 to 4 h [12]. There is also a very long terminal half-life of NIC attributed to the slow release of NIC from many tissues of the body. The volume of distribution of NIC is very large and reflects avid uptake and localization of lipid soluble NIC by body tissues [45]. The metabolic clearance of NIC is high, ranging from 1.3 to 2.5 l/min [12]. NIC often exhibits marked interindividual variability in the metabolic clearance, secondary to changes in hepatic blood flow [45].

The NIC concentrations in the smoker's plasma during the day typically range from 20 to 40 ng/ml [12]. Arteriovenous differences during cigarette smoking have been reported, with arterial levels exceeding venous levels by from sixfold to tenfold. There are considerable individual differences in the NIC concentrations in the plasma. These individual differences may be, at least in part, caused by different intake of NIC from a cigarette among people. It is well known that smokers can manipulate their intake of NIC. Many factors like the number of puffs, the intensity of puffing, the depth of inhalation, changing the puff volume, and the extent of dilution with room air can influence NIC intake, and, consequently, the NIC concentrations in the plasma [39]. NIC protein binding is only approximately 5%, which is too low to be biologically important [45].

The main organ for NIC excretion is the kidney. Renal clearance of NIC accounts for 2 to 35% of total NIC clearance [93]. Renal excretion of NIC is a pH-dependent process [80]. When urine pH is less than 5, total plasma clearance of NIC rises, reflecting an increase in renal clearance [71]. Urinary acidification

results in 18% increase in the intake of NIC from cigarette smoking [6]. Another important route for NIC excretion is saliva [73]. Sampling of saliva with measurement of NIC and its metabolites has been proposed as a convenient, non-invasive method of the NIC pharmacokinetics estimating [24, 95].

Metabolism of nicotine

Characterization of nicotine metabolism

Metabolism of NIC has been extensively examined *in vitro* and *in vivo*. *In vitro* models include use of intact cell, hepatocytes and perfused isolated organ systems. Among *in vitro* models, hepatic microsomal enzymatic system has become a popular and valuable model. Hepatic animal or human microsomes are incubated with NIC or its principal metabolite, cotinine (COT) under physiological conditions, and formation of NIC or COT metabolites is assayed in the samples after incubation by gas chromatography/mass spectrometry. The application of highly sensitive gas chromatography/mass spectrometry led to the identification and quantitation of new NIC metabolites as well as characterization of new pathways of NIC biotransformation. By using microsomes, it is possible to examine the role of each factor influencing NIC metabolism independently, and to determine dose-response relationships.

A number of *in vivo* studies concerning the NIC clinical pharmacology have been performed on animals. However, the animal studies have several important limitations and the question of whether the data obtained from NIC animal studies can be extrapolated to humans is open. The most important limitation is that NIC metabolism may differ among species [41, 44, 45, 63]. In a recent study of Tutka et al. [unpublished data], the significant differences in NIC metabolism were found among human, rabbit, and rat, confirming species variability in NIC metabolism. The study showed that a profile of NIC metabolism in rabbit was different from that of the rat. In contrast to rats, rabbits seem to be a good model for studying human NIC metabolism. Recently, an African green monkey model has been developed for evaluation of an effect of long-term NIC treatment on NIC metabolism [76].

The metabolism of NIC has been extensively studied in *in vivo* adult human studies. These studies have been based on the fact that the pharmacokinetics of NIC administered intravenously is comparable to that observed after absorption in the lungs during cigarette smoking. However, the presence of NIC and COT in the bodies of smokers does not allow us to do a pharmacological study without an addition of isotopes to the administered NIC or COT. Some earlier studies have been performed using intravenous injections of ^{14}C-labeled racemic NIC [43], but most recent studies have used intravenous infusions of deuterium-labeled NIC or deuterium-labeled COT at doses similar to those consumed during regular cigarette smoking [7, 10]. In such studies, after administration of radiolabeled analogs of NIC or COT, the concentrations of natural and labeled NIC and/or COT in the plasma and urine of the subjects are measured.

Over the past several years, the new methods have been developed to study the NIC kinetics and metabolism. Some studies have used NIC administered orally [22, 28, 77]. Before reaching the systemic circulation, oral NIC is extensively metabolized during first pass through the liver and the oral bioavailability of NIC is only 30% to 40% [10]. However, the combined administration of oral NIC with CYP2A6 inhibitor, such as methoxsalen (8-methoxypsoralen), increases the bioavailability of NIC to more than 60% [77].

Currently available techniques of molecular biology allow us to clarify the role of genetic factors in pharmacology of NIC and mechanisms leading to NIC addiction. Actually, the majority of the genetic studies focus on the characterization of the CYP2A6 gene polymorphism, and on determining the relationship between the phenotype of NIC metabolism and the genotype of the CYP2A6 gene. Genetic studies on smoking have been performed in twins and showed significant genetic contributions to the development of tobacco addiction and the likelihood of smoking cessation [19]. However, twin studies with larger group of subjects are necessary to confirm the role of genes in NIC pharmacology and tobacco addiction. Perhaps, when *in vivo* studies performed on a large number of individuals confirm the relationship between the phenotype of NIC metabolism and the genotype of the CYP2A6 gene, molecular studies will be used to determine the risk of lung cancer.

The main organ metabolizing NIC in the human body is the liver. It has been determined that 80% of NIC absorbed by a smoker is metabolized by *C*-oxidation to COT [11]. Metabolism of NIC to COT is an NADPH-dependent process [45]. The first step, the

conversion of NIC to the nicotine-iminium ion, is catalyzed by cytochromes P-450, and numerous studies have indicated that CYP2A6 enzyme is responsible for this reaction [56, 61]. The second step, the metabolism of the iminium ion to COT, is mediated by cytosolic aldehyde oxidase, although a microsomal enzyme may also be involved [64]. CYP2B6 may also inactivate NIC to COT but it has lower affinity and variable expression in human liver [90]. Pharmacokinetics and metabolism of COT are discussed in the next section.

Recent advances in analytical techniques have resulted in the discovery of a number of NIC metabolites and pathways of its biotransformation. These pathways include *N*-oxidation, *N*-demethylation, and glucuronidation [45]. *N*-oxidation is believed to be an important route of NIC biotransformation. The profile of NIC metabolism in human indicates that approximately 4% of NIC is metabolized to nicotine-1'-*N*-oxide [11], which is the main product of *N*-oxidation. The nicotine-1'-*N*-oxide formation occurs through a reaction catalyzed by flavin-containing monooxygenase [20], a flavoprotein found in many tissues. It has been suggested that nicotine-1'-*N*-oxide administered intraperitoneally in rabbit could be reduced back to NIC and could represent a reservoir for sustained generation of NIC [30]. Therefore, it is reasonable to suppose that nicotine-1'-*N*-oxide may influence the pharmacokinetics of NIC in humans. In *in vitro* experiments, the nicotine-1'-*N*-oxide formation in humans seems to be similar to the formation in rabbit but not in rat. In rat, nicotine-1'-*N*-oxide is the major metabolite of NIC [Tutka et al., unpublished data]. Although nicotine-1'-*N*-oxide is generally regarded as non-toxic, it has been proposed that it may be converted to the tobacco-specific nitrosamines, which are thought to play a major role in tobacco-related carcinogenesis [88].

The main product of *N*-demethylation of NIC is nornicotine [45]. The majority of nornicotine excreted by cigarette smokers is derived from NIC metabolism even though up to 40% of nornicotine may come from tobacco *per se* [11]. Nornicotine has been found in small amounts in human urine [11]. It has also been isolated from the urine of a number of animal species after NIC administration [55]. Crooks et al. [23] have demonstrated the relatively high levels of nornicotine in the rat brain after peripheral NIC injection. They have suggested that nornicotine is formed *via* oxidative *N*-demethylation of NIC locally in the brain. The functional role of nornicotine in the brain has not been determined yet although it is known that nornicotine exerts some pharmacological and toxic activity, contributing to the neuropharmacological effect of NIC [35].

Another minor metabolite of NIC is β-nicotyrine [78]. Studies on β-nicotyrine, employing cytochrome P450s-rich Clara cells isolated from rabbit lung, have shown that β-nicotyrine is bioactivated in an NADPH-dependent reaction to form pneumotoxic metabolites. Recent studies on the *in vivo* metabolic fate of β-nicotyrine in rabbit have identified *cis*-3'-hydroxycotinine as the principal urinary metabolite of β-nicotyrine [49]. The pharmacological activity of β-nicotyrine has not been clearly determined.

Another pathway of NIC biotransformation is *N*-glucuronidation. NIC glucuronide accounts for 4% of the total NIC metabolites in human [11]. The involvement of UDP-glucuronosyltransferase1A1 and 1A9 as well as 1A4 isoforms in NIC glucuronidation has been suggested, although the contributions of each isoforms have not been determined conclusively [42, 59].

Relatively little work has been focused on the involvement of other organs in NIC metabolism. NIC also appears to be metabolized, at least to a small extent, in the lung and kidney [45]. No detailed data are available concerning biotransformation of NIC in the brain. NIC metabolism in the brain appears to be of importance because of many neuropharmacological effects that result from NIC exposure. Some studies demonstrated the presence of NIC metabolites in the brain. It is unclear, whether NIC metabolites in the brain originate from NIC biotransformation or from uptake of peripheral NIC metabolites into the brain [23]. The experiments with the use of radiolabeled metabolites would be beneficial to elucidate the origin of brain NIC metabolites.

Pharmacokinetics and metabolism of cotinine

Pharmacokinetics and metabolism of COT has been examined in rodents and humans. Similarly to NIC metabolism, there are the significant species differences in COT metabolism among human, rabbit, and rat [Tutka et al., unpublished data]. For example, any cotinine metabolism in the rat liver microsomes has not been observed. Moreover, there has been quantitative differences in the formation of 3'-hydroxycotinine, 5'-hydroxycotinine, cotinine-N'-oxide, and norcotinine between human and rabbit.

COT is the principal metabolite of NIC in humans. The agent has some pharmacological activity [40].

A summary of its pharmacokinetcs in humans is shown in Table 1. COT can be given orally and its bioavailability is close to 100% [29]. The elimination half-life of COT averages about 17 h [7, 94], and COT is eliminated over a much longer period of time compared with NIC. The plasma concentrations of COT are highly correlated with the COT concentrations in the saliva or urine [4]. Thus, saliva and plasma COT concentrations can be used interchangeably [4, 95]. The plasma COT concentrations provide the best available measure of human environmental tobacco smoke exposure [4, 83]. Because the clearance of COT is highly correlated with the clearance of NIC [11], it has been proposed that oral clearance of COT could be a good marker of NIC clearance [95].

While there are numerous studies on NIC metabolism, the metabolism of COT has been less well characterized. COT is metabolized to 3'-hydroxycotinine, which is the most abundant metabolite of NIC, accounting for 38% of its all urinary metabolites in humans [11]. CYP2A6 is the primary enzyme that catalyzes the conversion of cotinine to 3'-hydroxycotinine [58]. Its elimination half-life is generation-limited and is similar to that of cotinine. 3'-hydroxycotinine does not exert NIC-like cardiovascular effects [9]. Recent *in vitro* studies [Tutka et al., unpublished data] have shown that the major product of COT metabolism in human liver is not 3'-hydroxycotinine but 5'-hydroxycotinine, which accounts for more than 50% of all microsomal metabolites. While a large body of data exists supporting the importance of 3'-hydroxycotinine, none of the previous studies measured the level of 5'-hydroxycotinine. The lack of an *in vivo-in vitro* correlation in the pattern of COT metabolism in human may be explained by interconversion of COT metabolites. According to Castagnoli et al. [21], who have investigated NIC metabolism in rabbit, 3'-hydroxycotinine could be formed from 5'-hydroxycotinine. Perhaps, the same route also exists in humans, but specific analyses are necessary to confirm it.

Of note is that the ratio of 3-hydroxycotinine/COT measured in plasma and saliva is highly correlated with the oral clearance of NIC. Therefore, Dempsey et al. [28] have proposed that this ratio is useful as a noninvasive marker of the rate of NIC metabolism and can be of use in smoking and addiction studies.

Little research has been done on other minor metabolites of COT. In smokers, COT and 3'-hydroxycotinine undergo conjugation reaction and their glucuronide conjugates are excreted in the urine [17, 18]. As shown by Benowitz et al. [11], the excretion of COT glucuronide and 3'-hydroxycotinine glucuronide as a fraction of the systemic NIC dose accounts for 13 and 7%, respectively. In the same study, a high correlation in the extent of *N*-glucuronide formation between NIC and COT within individuals was shown, indicating that the same enzyme was involved in *N*-glucuronidation of both agents. The conjugation of 3'-hydroxycotinine was unrelated to that of NIC and COT, and most likely, a different enzyme was involved in conjugation of 3'-hydroxycotinine than in conjugation of NIC and COT [11]. However, these results have not been confirmed *in vitro* by Kuehl and Murphy [42] who, using human liver microsomes, have suggested that the same enzyme(s) catalyze *N*-glucuronidation of NIC, COT and 3'-hydroxycotinine. Large hepatic interindividual variations in *N*-glucuronidation of NIC and COT have been reported by Ghosheh and Hawes [34]. COT is also metabolized to small amounts of cotinine-*N*'-oxide and norcotinine [45], which account for 2.4 and 2% of the NIC systemic dose, respectively [17].

Factors influencing nicotine and cotinine metabolism

NIC metabolism results in the generation of various metabolites, and individual, racial, and species variability in the profile and the rate of NIC metabolism have been well documented. In experiments using radiolabeled NIC infused intravenously to subjects, considerable individual differences in the clearance of NIC and the percentage of NIC conversion to COT have been demonstrated [7]. The excretion of NIC, COT, and 3'-hydroxycotinine, measured on the basis of 24-h urine collection, significantly varies among smokers [11]. Also, the extent of NIC and COT glucuronidation is different among individuals [11, 17]. It is known that only 25% of young people experimenting with cigarettes become tobacco addicts [31]. Large individual variability in the kinetics and metabolism of NIC could, at least partially, explain individual differences in susceptibility to NIC addiction.

Racial differences in NIC metabolism are indicated by racial differences in the plasma COT levels between black and white cigarette smokers [85]. Black smokers have the higher plasma COT levels than white smokers. The higher COT levels per cigarette in blacks are due to greater NIC intake per cigarette,

most likely through more intensive inhalation in blacks than in whites [68]. Recently, it has been found that the clearance of COT, the fractional conversion of NIC to COT, and the metabolic clearance of NIC to COT are lower in blacks than in whites. Furthermore, black smokers conjugate NIC and COT more slowly compared with white smokers. In summary, blacks metabolize NIC more slowly than whites *via* COT pathway. This may explain greater incidence of tobacco-related lung cancer in black in comparison with white smokers [75]. Similar studies have shown slower total clearance of NIC and COT, and the metabolic clearance of NIC in Chinese-Americans compared to Latinos and whites [13]. Such differences in NIC and COT metabolism appear to be consistent with the epidemiological data, which have shown lower incidence of lung cancer in Chinese-Americans.

Individual and racial differences in the metabolism of NIC may be a result of genetic, environmental, and developmental host influences. Because of the importance of CYP2A6 in NIC metabolism, it has been suggested that the CYP2A6 genotype influences the individual differences in NIC metabolism and susceptibility to smoking addiction [60, 69, 84].

Cotinine formation from NIC in human liver microsomes is correlated with CYP2A6 levels and coumarin 7-hydroxylation [61]. The large individual and ethnic variability in levels of CYP2A6 mRNA [57] and CYP2A6 protein [92], as well as in coumarin 7-hydroxylase activity observed in humans, suggests that the rate and the profile of NIC metabolism may be genetically determined [56] and related to the genetic polymorphism of CYP2A6 gene [61]. For this gene, two catalytically inactive variants, CYP2A6v1 and CYP2A6v2 have been reported [33]. The presence of the CYP2A6v1 and CYP2A6v2 alleles significantly decreased the number of cigarettes consumed by smokers [69], although this relation has not been confirmed in another study [50]. The frequency of the mutated alleles varies considerably among different ethnic populations [70]. The frequency of both alleles is low in European populations and very few poor metabolizers have been described in these populations [66].

A homozygous whole deletion allele of CYP2A6 gene has been reported [62]. The whole deletion of CYP2A6 gene could be responsible for the poor metabolism of NIC to COT in humans. Nakajima et al. [60] have measured the plasma concentration of NIC and COT in healthy subjects after each smoked one cigarette or chewed one piece of NIC gum. One subject showed no detectable COT level when smoking and the lowest COT level when receiving NIC gum. This subject was regarded to be a poor metabolizer of NIC and was found to carry the homozygous whole deletion type of CYP2A6 gene. In another study, an ethnic-related difference in the allelic frequency of the whole deletion allele of CYP2A6 gene has been observed [67]. A relatively high allele frequency (15–20%) of the CYP2A6 gene deletion has been found in Asian population [67].

A genetic deficiency in NIC metabolism caused by defective mutations in CYP2A6 may be associated with a lower risk to become tobacco dependent [69]. It is well known that smoking is strongly linked to lung cancer [25]. CYP 2A6 has been reported to activate a number of harmful procarcinogens, including tobacco-specific nitrosamines and aflatoxin B1, contained in cigarette smoke [91]. The individuals who carry a mutation or deletion in the CYP2A6 gene may have a decreased risk of tobacco-related cancers [60]. Therefore, it has been proposed that CYP2A6 inhibition could be used to reduce dependent users' rate of smoking and exposure to procarcinogens contained in cigarette smoke or as a part of a step-care reduction of smoking, leading to cessation [77]. Methoxsalen (8-methoxypsoralen), the drug used in the treatment of psoriasis, has been reported as a potent inhibitor of CYP2A6. Methoxsalen potently inhibits first-pass metabolism of orally administered NIC [77] and is taken into account as a potential drug in the therapy of NIC addiction. However, methoxsalen is also an inhibitor of CYP1A2 and, perhaps, other P450 forms and the lack of selectivity could speak against its use in the treatment of tobacco addiction. Furthermore, methoxsalen, inhibiting CYP2A6 and CYP1A2 enzymes, may affect metabolism of certain clinically used drugs metabolized *via* CYP2A6 or CYP1A2. Finally, the safety of methoxsalen during long-term use has not been determined. Despite these limitations, the question whether CYP2A6 inhibitors can be useful in the therapy of NIC addiction is still open. The identification of new potent, safe, more specific and selective CYP2A6 inhibitors is a high research priority.

Could CYP2A6 genotype influence lung cancer susceptibility? Several case-control studies have been conducted to answer this question, but the studies gave conflicting results [50, 51, 66, 70]. The study by Loriot et al. [51] has confirmed neither a relation between genetically impaired NIC metabolism and ciga-

rette consumption nor any modification of lung cancer risk related to the presence of defective CYP2A6 alleles.

Smoking is another important factor that may influence the NIC metabolism. The first reports demonstrated that smokers metabolized NIC more rapidly than nonsmokers [37, 43]. These studies involved administration of very low doses of racemic NIC. Later, Benowitz et al. [7, 9], infusing intravenously deuterium-labeled NIC in smokers when they were smoking, showed that smokers metabolize NIC slower than nonsmokers. The mechanism responsible for the reduced NIC metabolism during smoking may involve decreasing the expression of CYP2A6 by NIC itself [76].

In another study, cigarette smoking significantly inhibited metabolic clearance of NIC. This inhibition was not related to COT and carbon monoxide [8, 95]. Another factor that may influence the differences in NIC metabolism is smoking mentholated cigarettes [54]. There is evidence that many more black than white men smoke mentholated cigarettes [68]. Menthol cools the airways and might be associated with a greater volume or depth of inhalation [68].

NIC metabolism may be affected by some drugs [72, 87]. For example, human hepatocytes from individuals treated *in vivo* with phenobarbital show higher-than-normal NIC oxidation rates on hepatocyte harvest [87]. On the other hand, no association has been found between the drug histories and the microsomal levels of CYP2A6 protein and CYP2A6 activity in subjects [61].

There are no available data concerning the influence of diet on NIC metabolism, although several studies have suggested a relation between a coffee consumption [16, 45] or a high-protein diet [45, 46] and NIC kinetics.

The detailed NIC pharmacokinetics and quantitative pattern of the generated metabolites in newborns have not been definitively determined. The elimination half-life of NIC in newborns is three to four times that of adults, whereas the half-life of COT in newborns is essentially the same as that in adults [26, 32]. Newborns, compared with adults, have prolonged elimination of NIC, but similar elimination of COT, 3'-hydroxycotinine, or the conjugated metabolites [26]. The enzymes involved in NIC and COT metabolism in newborns have not been well described. Dempsey et al. [26] have suggested that newborns might be deficient in CYP2A6 or have a fetal-newborn form of CYP2A6 that has an altered affinity for NIC but not for COT. It is likely that the differences in the pharmacokinetics and metabolism of NIC between newborns and adults are due to other than CYP2A6 enzymes.

Cigarette smoking during pregnancy has long been associated with adverse pregnancy outcomes in the mother and newborn. Little is known about NIC metabolism during pregnancy and existing data are controversial. In *in vitro* studies, any significant differences in the metabolic profile of NIC have not been found between pregnant and non-pregnant rabbits [82]. On the other hand, *in vivo* human studies have shown that the excretion of NIC metabolites in the urine of passive and active smokers rises with gestation [52]. In the study using transdermal NIC replacement, Wright et al. [89] have found that the salivary levels of NIC in pregnant women are consistent with those seen in non-pregnant women. The salivary COT levels are significantly lower in pregnant than non-pregnant women. Wright et al. have concluded that NIC is less rapidly metabolized in pregnant than non-pregnant women. Recently, it has been reported that NIC and COT clearances during pregnancy are significantly increased with no change in the fractional conversion of NIC to COT [27]. The change in NIC clearance may be due to the increased delivery of NIC to the liver as the result of the increase in hepatic blood flow observed during pregnancy.

There are no detailed data concerning the pharmacokinetics and metabolism of NIC in older patients. A high degree of individual variability in plasma NIC concentrations during smoking or NIC replacement therapy does not significantly change with advancing age [36]. On the other hand, it is possible that aging could decrease the volume of distribution of NIC because it is primarily distributed in lean body mass, which decreases with age. It has been suggested that in older patients, the changes in volume of distribution could influence the plasma NIC concentrations after absorption from rapid NIC delivery systems, such as NIC gum or nasal spray [36].

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