Overview: Cigarette smoking is responsible for numerous health problems, including cancer and cardiovascular and pulmonary disorders. It is the leading cause of preventable deaths worldwide. Nicotine is primarily responsible for the highly addictive properties of cigarettes. In humans, nicotine is mainly inactivated into cotinine, and the hepatic enzyme cytochrome P450 2A6 (CYP2A6) mediates the majority of this reaction. CYP2A6 also can metabolically activate tobacco-specific nitrosamines into their carcinogenic forms. The gene encoding CYP2A6 is highly polymorphic, and individuals with reduced- or loss-of function alleles have significantly slower rates of nicotine metabolism. Smokers are known to regulate their nicotine intake to maintain brain and plasma levels; thus, genetic variations in CYP2A6 have been hypothesized to influence smoking behaviors. This article summarizes studies examining the role of CYP2A6 genetic variation on nicotine pharmacokinetics and smoking. Studies have associated slow CYP2A6 metabolism with an altered risk of becoming dependent, lower risk of being a smoker, lower cigarette consumption, and lower risk of lung cancer. Furthermore, this article will review how genetic variability in CYP2A6 may have clinical implications in smoking cessation. CYP2A6 activity may be a useful predictor of the efficacy of nicotine replacement therapy because it may affect the nicotine levels derived. In addition, CYP2A6 inhibitors may have utility in smoking reduction and cessation by mimicking the phenotypes of slow metabolizers.

CIGARETTE SMOKING remains one of the leading causes of preventable disease and death. According to the World Health Organization, tobacco smoking is responsible for the deaths of approximately 5 million people each year. Despite increasingly strict tobacco control policies and the numerous adverse health effects associated with cigarette smoking, approximately 21% of adults in the United States are smokers. Cigarette smoking has substantial economic affects, with costs estimated at $167 billion annually as a result of productivity losses and health care expenditures.2 The highly addictive property of cigarettes is mediated by nicotine, the primary psychoactive substance in tobacco smoke that establishes and maintains dependence.3 Nicotine inhaled from a puff of cigarette smoke is rapidly absorbed by the pulmonary capillaries and reaches the brain in 10 to 20 seconds.4 Thus, the effects of nicotine are experienced immediately, and smokers must smoke regularly because of the relatively short half-life of nicotine (1–2 hours).4 Dependent smokers are known to regulate their intake to maintain levels in the brain and blood. Smoking can be altered by changing the nicotine content in cigarettes or by manipulating the rate of renal nicotine excretion.5 Thus, individual variations in the rate of nicotine metabolism may be an important determinant of smoking behaviors.

For humans, the main route of elimination for nicotine is by way of hepatic metabolism. Approximately 70% to 80% of nicotine is inactivated to cotinine,6 and approximately 90% of this reaction is mediated by the hepatic enzyme cytochrome P450 2A6 (CYP2A6).4 Cotinine is mostly further metabolized to trans-3-hydroxycotinine (3HC); this reaction is mediated entirely by CYP2A6.4 In addition, CYP2A6 can bioactivate N-nitrosamines, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK), one of the most potent and abundant procarcinogens in tobacco smoke.6,7

This article will describe the genetic variability of CYP2A6 and of nicotine metabolism, and how they have been implicated in smoking behaviors. The ways in which CYP2A6 activity may influence the efficacy of nicotine replacement therapy (NRT) by affecting the nicotine levels derived from these sources also will be discussed. The use of CYP2A6 inhibitors to mimic or phenocopy slow metabolizers as a novel method of smoking reduction and cessation also is explored.

VARIABILITY IN CYP2A6 AND NICOTINE METABOLISM

Large interindividual variations in CYP2A6 activity have been reported.8 Human liver microsomes were found to have a more than 100-fold variation in coumarin 7-hydroxylation, a common substrate probe for CYP2A6 activity.9 Variations in CYP2A6 activity and rates of nicotine metabolism also have been reported across ethnic groups.8,10 For example, liver microsomes from Japanese donors were found to have lower levels of CYP2A6 protein and activity, compared with those from whites.11 Furthermore, following intravenous infusions of isotope-labeled nicotine and cotinine, nicotine metabolism was found to be 18% slower for Asians12 and 13% slower for blacks13 compared with whites; cotinine metabolism was found to be 31% slower for Asians12 and 32% slower for blacks.13 Although other factors such as diet, gender, smoking status, and therapeutic compounds may contribute to the variation in activity through induction or inhibition,14 this variability has mainly been attributed to genetic polymorphisms in CYP2A6.
CYP2A6 is located in the CYP cluster (CYP2A, CYP2B, CYP2E, CYP2G, CYP2S, CYP2T) on chromosome 19q13.2, spanning approximately 6 kb with 9 exons encoding 494 amino acids. CYP2A6 is highly polymorphic, with 23 numbered alleles and numerous uncharacterized single nucleotide polymorphisms identified so far (http://www.cypalleles.ki.se/cyp2a6.htm). Some alleles encode enzymes with complete loss of function (e.g., CYP2A6*2, CYP2A6*4), others reduce activity (e.g., CYP2A6*9, CYP2A6*12), and the gene duplication alleles (CYP2A6*1x2A, *1x2B) may increase activity. Individuals with CYP2A6 genetic variants generally have altered nicotine pharmacokinetics. For example, individuals homozygous for the gene deletion allele (CYP2A6*4) were found to have 3.6-fold higher systemic exposure following administration of oral nicotine (4 mg), compared with those having CYP2A6*1/*1. Furthermore, following nicotine administration, the urinary profile of individuals who express CYP2A6*4/*4 showed very little formation of cotinine, 3HC, or their glucuronide derivatives; however, these form the majority of the excreted metabolites for wild-type individuals.2

The distribution of CYP2A6 alleles also varies widely across ethnic groups (Fig. 1); this is primarily consistent with observed differences in nicotine metabolism rates.11-13 In general, reduced- or loss-of-function CYP2A6 alleles are more prevalent for Asians, although variations in CYP2A6 allele frequencies have been observed even among Asian ethnic groups (Fig. 1).10

**EFFECT OF CYP2A6 VARIATION ON SMOKING BEHAVIORS**

Smoking patterns are known to be affected by nicotine plasma levels. Because CYP2A6 slow metabolizers have higher and more prolonged levels of nicotine, they are predicted to smoke less frequently, thereby consuming fewer cigarettes to maintain nicotine levels, compared with individuals who metabolize CYP2A6 at a normal rate. Indeed, studies have found that CYP2A6 slow metabolizers consume less cigarettes (as indicated by self-report and biomarkers such as carbon monoxide levels) and smoke less intensely (as indicated by puff volume)8,10. In addition, CYP2A6 slow metabolizers were less likely to be smokers.8,10 One interpretation is that slow CYP2A6 activity enables individuals to have greater success with cessation, because reduced cigarette consumption has been associated with improved cessation outcomes for adults.15 Cessation is a difficult process, and smokers typically make multiple quit attempts before achieving long-term abstinence. We have found that CYP2A6 slow metabolizers experienced shorter smoking durations, suggesting that these individuals were able to quit sooner.16 In a case-controlled study examining current and former smokers, individuals with the CYP2A6*2 allele were found to be 1.75-times more likely to have quit compared with individuals without the allele.17

CYP2A6 slow activity also may be protective against the progression to becoming a “regular smoker” during acquisition stages. In retrospective studies, CYP2A6 slow metabolizers have reported earlier ages of smoking initiation; however, such findings are limited because of recall bias. Few studies have been conducted to prospectively follow adolescent groups — a period during which the majority of smoking experimentation and initiation occurs. One such study found that young adolescents with
reduced and loss-of-function CYP2A6 alleles have a higher risk (approximately 3-fold) of converting to nicotine dependence (as measured by the International Statistical Classification of Diseases and Related Health Problems, 10th revision); however, these adolescents consumed less cigarettes once they became dependent, a trend in agreement with studies of adults. Another study found that CYP2A6 slow metabolizers experienced slower rates of progression to higher levels of nicotine dependence (as measured by the modified Fagerström Tolerance Questionnaire); these same individuals also reported lower cigarette consumption among dependent smokers. Thus, the genetic factors regulating smoking behaviors are complex, and further examination of the role of CYP2A6 during different stages of smoking (i.e., initiation and cessation) is warranted.

CYP2A6 genetic variations resulting in reduced or deficient activity also have been associated with lower risk of lung cancer, and these studies have been reviewed extensively elsewhere. The protective effect of CYP2A6 slow activity toward tobacco-related lung cancer may be associated with both the lower risk of being a smoker and reduced cigarette consumption if one does smoke. It is possible that CYP2A6 genetic variants with reduced- or loss-of-function may be less efficient at bioactivating NNK, a procarcinogen that can be activated by alpha-hydroxylation to a reactive metabolite capable of forming DNA adducts, with CYP2A6 being one of the enzymes involved in this reaction. Alternatively, NNK can be detoxified by conversion to 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanol (NNAL) and subsequent conjugation to form NNAL-glucuronide. The effects of CYP2A6 genetic variants on NNK metabolism has not been tested directly but would be of interest to better understand the role of CYP2A6 on cancer risk.

It should be noted that not all studies regarding the role of CYP2A6 in smoking behaviors and cancer have been in agreement. One meta-analysis failed to find any association between CYP2A6 genotype and smoking status or cigarette consumption, although another meta-analysis reported a modest effect of CYP2A6 on smoking cessation and cigarette consumption. Some studies have failed to observe any association between CYP2A6 genotype and cancer risk. This may be the result of several factors, including the lack of statistic power or population stratification, the presence of unidentified CYP2A6 alleles among individuals designated as wild-type, and variations in definitions of smoking and cancer phenotypes.

CYP2A6 ACTIVITY AND EFFICACY FROM NRT

NRT typically increases the odds of quitting 1.5- to 2-fold; however, there is large inter-individual variability in response, and few individuals maintain long-term abstinence. Individuals with CYP2A6 genetic variants are predicted to respond differently to NRTs as a result of their slower rates of nicotine metabolism. CYP2A6 slow metabolizers achieve higher nicotine plasma levels while using the transdermal nicotine patch compared with individuals who metabolize CYP2A6 at normal rates. Interestingly, CYP2A6 slow metabolizers on nicotine spray attained similar nicotine levels to those attained by normal metabolizers by reducing the number of sprays used per day. Using the 3HC/COT ratio, a validated biomarker of CYP2A6 activity, CYP2A6 slow metabolizers were found to have higher abstinence rates on the transdermal patch compared with normal metabolizers; no difference was found for those using nasal spray consistent with their similar nicotine plasma levels. Thus, CYP2A6 activity may be more important in therapeutic outcomes for NRT, wherein doses are constant and nontitratable (e.g., transdermal patch), compared with cessation techniques in which dosages can be adjusted for differing rates of metabolism and need (e.g., nasal spray). This may have important implications because smoking cessation treatments (such as dose or type of NRT) could be recommended based on an individual’s CYP2A6 genotype or phenotype to maximize therapeutic effects.

USING CYP2A6 INHIBITORS FOR THE TREATMENT OF TOBACCO DEPENDENCE

Based on the effect of CYP2A6 genotype on nicotine metabolism and smoking behaviors, manipulation of CYP2A6 activity to mimic slow metabolizers may be useful in reducing smoking and aiding cessation. Methoxsalen is a CYP2A6 inhibitor that has been shown in pilot studies to inhibit nicotine metabolism and reduce smoking.

CYP2A6 inhibitors may be used to enhance the efficacy of NRTs (e.g., gum, patch), which are only modestly effective, because they provide approximately 50% of the plasma nicotine levels obtained from smoking. Furthermore, the nicotine levels attained from NRT are highly variable between individuals as a result of differences in metabolism rates. Thus, concurrent administration of CYP2A6 inhibitors with NRT is predicted to enhance efficacy by increasing nicotine levels and prolonging its duration of action. Concurrent administration also could make the dosing regimen more predictable by reducing intra- and inter-individual variation in nicotine metabolism. One study has found that nicotine plasma levels were significantly higher for individuals who received methoxsalen plus nicotine gum compared with placebo plus nicotine gum (15.3 vs. 10.1 ng/mL; p < 0.01).

In addition, NRT is not available in a form that can be ingested orally, which is the preferred route of administration because of the high first-pass metabolism that breaks down 70% to 80% of the nicotine before it reaches systemic circulation. Providing higher doses of oral nicotine is not feasible because of the resulting gastrointestinal irritations. As such, CYP2A6 inhibitors may facilitate the use of oral nicotine as a form of NRT. One study has found that methoxsalen given with 4 mg of oral nicotine resulted in higher plasma nicotine levels and reduced smoking (as indicated by decreased breath carbon monoxide levels, numbers of puffs, number of cigarettes smoked, and increased latency to next cigarette) during ad libitum smoking.
GENETIC VARIABILITY IN CYP2A6 AND SMOKING

In addition to reducing smoking, CYP2A6 inhibition may reduce the bioactivation of procarcinogens such as NNK. One study found smokers who were given methoxsalen for more than 3 days while maintaining smoking rates had significantly more NNK metabolized to the inactive NNAL-glucuronide compared with baseline in the absence of inhibitor.27 This suggests inhibition of CYP2A6 resulted in the rerouting of NNK from its mutagenic alpha-hydroxylation pathway to a detoxifying glucuronidation pathway. Furthermore, studies in mice demonstrated methoxsalen was able to prevent the formation of NNK-induced lung adenomas28,29

CONCLUSION

Despite the numerous adverse health consequences associated with smoking, vast numbers of people continue to smoke. Although many smokers express a desire to quit, it is a difficult process because of the highly addictive properties of nicotine delivered by cigarette smoking. Genetic variability in CYP2A6, the main nicotine-metabolizing enzyme, has been implicated in smoking. There have been substantial advances in the identification and characterization of new CYP2A6 genetic variants in recent years, which hopefully will allow future studies to better clarify the role of CYP2A6 in smoking behaviors. Further research must be conducted to examine how the CYP2A6 genotype may influence response to NRT and how this information could be used to tailor individual treatment to maximize cessation success. In addition, more research is required to test the potential benefits and feasibility of using CYP2A6 inhibitors in smoking cessation treatment. A better understanding of the role of variability of CYP2A6 in nicotine kinetics and smoking behaviors may lead to more effective smoking prevention and treatment strategies.

Funding was received from Canadian Institute for Health Research (CIHR) MOP53248 grant, NSERC CGS-D Postgraduate Scholarship (MKH), and a Canada Research Chair in Pharmacogenetics (RFT).

REFERENCES


