Decreasing smoking behaviour and risk through CYP2A6 inhibition

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The consequences of tobacco dependence are both health related and economic. Novel treatment approaches are needed to offer alternatives to patients and to improve treatment outcomes. We review concepts and selected recent discoveries in the area of treatment, with a specific orientation towards drug development. Current treatments are outlined and we highlight new strategies that are based on the manipulation of cytochrome P450 2A6 (CYP2A6) activity, which is responsible for the metabolism of nicotine. The clinical implications of *CYP2A6* polymorphisms have been linked to a decreased risk of tobacco dependence, a decrease in number of cigarettes smoked and reduced risk of tobacco-related cancers. Further, we discuss a range of models for proof-of-concept studies for new treatments to alleviate tobacco dependence.

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▼ Tobacco smoking is the single most preventable cause of death in the world today. Approximately 1.2 billion people worldwide are known to smoke tobacco daily, resulting in the global consumption rate of 5.6 trillion cigarettes per year at the end of the 20th century [1,2]. Currently, an estimated 4.2 million people die annually from tobacco-related diseases, and this number is predicted to approach 10 million by the year 2020 [1].

Smoking prevalence and reason for concern

The health consequences of smoking include respiratory, cardiovascular and cerebrovascular disorders and cancer. The most well-established association between smoking and disease is that for cancer (i.e. cancers of the lung, oral cavity, pharynx, pancreatic, kidney and urinary tract), which is also the most widespread smoking-related disease. It is estimated that smoking is the leading cause of death from cancer, accounting for 15% of all cancers, and is responsible for 80–90% of all lung cancers [3–5]. This is because cigarette smoking (particulate phase, vapour phase and tobacco smoke) typically results in exposure to a complex combination of more than 4000 chemicals, of which more than 100 are established mutagens and carcinogens [6–8].

Smoking is an epidemic in both wealthy and developing nations. The consequences of smoking are both health related for the individual and economic for society. In the United States, there are more than 400,000 deaths from smoking each year, costing the nation tens of billions of dollars annually [9,10]. A higher mortality rate is expected in developing countries where two-thirds of the predicted ~10 million smoking-related deaths worldwide in the year 2020 are expected to occur [9].

Smoking is a regulated behaviour

Smoking is a complex regulated behaviour [11] that is influenced by both genetic and environmental factors [12]. Genetics explains ~50% of variation in the initiation of smoking, ~70% of the variation in maintenance and more than 80% of the variation in number of cigarettes smoked [12,13]. The dangerous components of tobacco smoke (carcinogens, carbon monoxide etc.) play a key role in the etiology of smoking-induced disorders. Nicotine, however, is the primary component of tobacco responsible for dependence on cigarette smoking [11,14,15]. Nicotine-dependent individuals regulate their smoking to maintain nicotine levels in their brain [14,16,17].

Dependence on nicotine is related to the rapid rise of nicotine concentrations in the arterial plasma after cigarette smoking. This is because nicotine delivered via smoking rapidly enters the pulmonary rather than the portal or venous circulation. Once nicotine is absorbed from the alveolar capillary bed of the lung, it is pumped directly to the brain by the left ventricle of the heart. Nicotine reaches the brain an estimated 7–19 s after inhalation [16,18].

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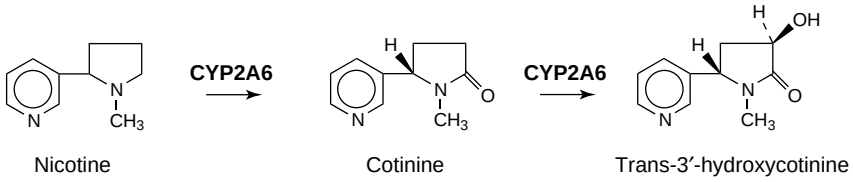

Figure 1. Nicotine metabolism by CYP2A6.

This arterial nicotine concentration 'spike' is estimated to be in the order of 10-fold greater than the corresponding spike in the venous nicotine concentration. The rapid delivery of nicotine to the brain causes the nicotinic receptors in the brain to experience a unique pattern of exposure, which is associated with strong drug reinforcement [19].

As the half-life of nicotine is short at 1–2 h, smokers continually titrate their smoking levels to ensure that brain (and plasma) levels of nicotine are maintained within the individual's target concentration [14]. Smokers have been shown to smoke low-yield nicotine cigarettes more intensely than high-yield cigarettes to titrate the level of nicotine to reach specific target concentrations in the brain [20]. Over the day, repeated smoking allows plasma concentrations of nicotine rise and reach the desired or titrated concentration goal and plateau for the individual. Overnight, when smoking stops, the fall in plasma nicotine concentrations and associated withdrawal are important determinants of the need to resume smoking the next day.

Current treatment for tobacco dependence

Nicotine replacement therapy (NRT) is the most commonly used pharmacological approach for treating nicotine dependence. NRT provides smokers with nicotine so that they can partly overcome their physical and psychological dependence on nicotine and cigarette smoking [21]. In this way, smokers still receive nicotine but the method of delivery is safer than tobacco smoking. Common forms of NRT include nicotine gum, transdermal patches, inhalers, lozenges and sprays. Studies using these forms of NRT indicate that rapid increases in nicotine concentration in the brain are not necessary to alleviate most withdrawal symptoms [11]. Tolerance of and physical dependence on NRT are rarely seen because this approach provides nicotine doses that are lower and delivered more slowly than those provided by cigarette smoking [11,22]. NRTs that are currently available have been helpful for many smokers who are attempting to quit, but some patients cannot tolerate these delivery systems because of side effects or simply find them inconvenient or ineffective.

Bupropion, a non-nicotine pharmacological agent that was first marketed as an antidepressant, has been shown to be effective for smoking cessation [23]. It is currently approved and used for the treatment of tobacco dependence in more than 50 countries worldwide [24]. Bupropion is well tolerated, but side-effects such as nausea, insomnia and dry mouth have been reported and it is contraindicated in patients with a risk of seizures. Concerns over the safety of bupropion, including possible incidences of serious adverse events or death, have been raised but no causal relationship has been established [24].

Market exists for novel smoking-cessation strategies

Evidence has shown that stopping smoking can substantially reduce mortality risk, even among long-term smokers [25]. Because of the high smoking-related mortality rate and the highly publicized health consequences of smoking, the smoking cessation market is large with a potential to be expanded. Approximately 35% of smokers attempt to quit each year, but fewer than 5% are successful without some form of therapy (pharmacological or psychological) [26]. Existing NRTs can fail for several reasons: (1) the extent of nicotine replacement is inadequate compared to the nicotine obtained from cigarette smoking [27–30]; (2) NRT does not mimic the rapid rise and fall in the plasma nicotine concentration peak that is achieved by smoking cigarettes because plasma concentrations peak minutes and several hours after the administration of the gum and patch, respectively [27,29]; (3) there is wide variation in nicotine metabolism among individuals, and this results in a wide variation in nicotine concentration in plasma when standard NRT doses are taken [27,31]; (4) dermal or gastric irritation from the use of NRTs may limit their optimal use; and (5) other factors make important contributions to the maintenance of smoking (e.g. conditioned responses and environmental cues).

Novel treatment strategies based on genetic manipulation

In humans, the hepatic enzyme cytochrome P450 2A6 (CYP2A6) accounts for the majority (~90%) of the metabolic inactivation of nicotine to cotinine ([32]; Figure 1). CYP2A6 is a genetically polymorphic enzyme. To date, 17 allelic variants of *CYP2A6* have been identified (*1–*16 and the *1x2 gene duplication) and many have been characterized *in vitro* and *in vivo*. These variants can result in individuals with an impaired or enhanced ability to metabolize nicotine (i.e. an altered phenotype) [33]. The allelic variants also vary

in frequency across ethnic groups, and interethnic differences in the ability to metabolize CYP2A6 substrates may be attributed to variation in the frequencies of the different *CYP2A6* alleles between ethnic groups [34–36]. For example, African Americans have significantly reduced clearance of cotinine, fractional conversion of nicotine to cotinine, and metabolic clearance of nicotine to cotinine compared with Caucasians [37,38]. The metabolic ratio of cotinine to nicotine is also greater in Korean than in Japanese populations [39]. The clinical implications of these ethnic variations are important as the altered rate of metabolism of CYP2A6 substrates may affect smoking behaviour, risk of tobacco-related cancers and the use of nicotine replacement therapies to treat nicotine addiction and other disorders.

Public health implications of *CYP2A6* polymorphism

We have previously demonstrated that *CYP2A6* genetic variants that result in a decrease in nicotine metabolism *in vivo* (1) decrease the risk of smoking dependence, (2) decrease the number of cigarettes smoked, and (3) decrease the risk of tobacco-related cancers and mutations (Box 1).

1. Risk of smoking initiation and tobacco dependence

We hypothesized that individuals that have impaired nicotine metabolism (carriers of null or defective *CYP2A6* allele(s) or slow metabolizers [SMs]) would be protected from the risk of smoking initiation and becoming tobacco-dependent. First, they would use greater levels of nicotine when learning to smoke, thus increasing nicotine's primary aversive effects; and second, they would develop slower acquisition of withdrawal and tolerance. In our original epidemiological study, we found that tobacco-dependent subjects with inactive alleles had a lower risk of becoming tobacco dependent than subjects with two functional copies of the *CYP2A6* gene (*CYP2A6*1/*1* or extensive metabolizers [EMs]) [40]; however, there were issues with the genotyping assays used in this study [41]. Subsequent studies indicated that among Caucasians recruited and genotyped for *CYP2A6*2* and *CYP2A6*4*, significantly more tobacco non-dependents than tobacco dependents were SMs [35,42].

2. The number of cigarettes smoked and other smoking indices

Smokers adjust their smoking to maintain constant levels of nicotine on their plasma. We therefore hypothesized that SMs (i.e. those with one or fewer *CYP2A6* active alleles) would smoke less than EMs (i.e. those with two active *CYP2A6* gene copies) who, in turn, would smoke less than fast metabolizers ([FMs]; i.e. carriers of three or more active *CYP2A6* gene copies. In a prospective study of smokers, we recorded both self-reported smoking behaviour (current cigarettes/day and periods of heaviest smoking) and objective measures of smoking (nicotine and cotinine concentrations in plasma and CO concentrations in the breath). A comparative study looked at smokers who are carriers of *CYP2A6* null alleles (N=14), those homozygous for the wildtype allele (EMs; N=277) and those who are carriers of the *CYP2A6* duplication allele (N=5). This study showed that SMs smoked fewer cigarettes/day (13.5±2.3) than EMs (19.5±0.7) ($p<0.03$) to maintain equal plasma nicotine levels [42]. SMs also smoked fewer cigarettes during periods of heaviest smoking [43]. Furthermore, carriers of null alleles had significantly lower CO breath concentrations ($p<0.05$) (a measure of smoke exposure) and plasma cotinine concentrations ($p<0.05$) (a measure of the metabolism of nicotine to cotinine) than either EMs or carriers of the *CYP2A6* duplication gene variant [43]. Consistent with alterations in the metabolic inactivation of nicotine, several indices of smoking revealed that subjects who are SMs, EMs, and FMs displayed significantly different smoking patterns and intensities. For example, subjects with the *CYP2A6* duplication allele (FMs) smoked each cigarette more intensely, as indicated by a doubling of CO/cigarette ratio and greater increases in plasma nicotine concentration/cigarette, than wild-type homozygotes (EMs) or those with at least one null allele (SMs) [43]. Thus, it appears that those with duplicated alleles increase their smoking by increasing the intensity with which they inhale each cigarette, whereas those with null *CYP2A6* alleles decrease their smoking levels by smoking fewer cigarettes/day [43]. Consistent with our findings, Gu and colleagues [41] noted that individuals with the *CYP2A6*2* inactive allele started smoking on average three years later, smoked for a shorter duration and had an increased probability of quitting than individuals with two active alleles [42]. These data demonstrate a major role for *CYP2A6* gene variants in determining smoking patterns, with slower metabolizers smoking less than faster metabolizers.

Box 1.

Inhibition of CYP2A6 and slower nicotine metabolism has the following effects:

- Decreases risk of smoking initiation and dependence
- Decreases amount smoked
- Decreases risk of tobacco-related cancers and mutations

3. Risk of tobacco-related cancers and mutations

A critical review of tobacco components showed that tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (present at 5–200 ng/cigarette) are the classes of compounds that most strongly

Box 2.

Clinical implications of CYP2A6 inhibition as a treatment for tobacco dependence
- Facilitates oral nicotine as a form of NRT
- Reduces exposure to the harmful components of tobacco smoke
- Enhances the efficacy of nicotine gum, nicotine patches and other NRTs
- Decreases the load of carcinogens in the body

affect the risk of human cancers [7,44]. Two tobacco-specific pro-carcinogen nitrosamines that cause lung cancer, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN), are specifically activated to form carcinogens by CYP2A6 [45–47]. Individuals that are carriers of one or more defective *CYP2A6* alleles may be at lower risk of lung cancer development because (1) they have a decreased ability to bioactivate certain tobacco-specific pro-carcinogens; (2) they have a decreased risk of becoming tobacco-dependent; and (3) if tobacco-dependent, they smoke less, which is important considering that the amount of smoke exposure is exponentially related to cancer risk [48]. These factors suggest that for individuals who are carriers of one or more defective *CYP2A6* alleles, longer-term smoking is typically required for the development of tobacco-related cancers.

Clinical implications of CYP2A6 inhibition as a treatment for tobacco dependence

A treatment designed to mimic the genetic defect that inhibits the CYP2A6 enzyme of smokers *in vivo* offers a novel approach to the treatment of nicotine dependence. Inhibition of CYP2A6 is particularly attractive because this enzyme is not known to metabolize many clinically used drugs (except nicotine and SM-12502, which is a novel antagonist of platelet-activating factor receptor), and because some individuals and populations completely lack this enzyme (i.e. are homozygous for inactive alleles). CYP2A6 inhibition, and the decreased metabolism of nicotine to cotinine that ensues, should cause nicotine plasma levels to be maintained for longer periods of time, which may decrease the craving for nicotine. Nicotine-dependent individuals smoke cigarettes with the goal of maintaining target nicotine concentrations in the brain; thus, factors that promote a decrease in nicotine clearance (such as the inhibition of nicotine metabolism) should downregulate smoking behaviour. The clinical implications of CYP2A6 inhibition as a treatment for tobacco dependence include: (1) facilitating oral nicotine as a form of NRT, (2) reduced exposure to dangerous chemicals in smoke, (3) enhanced efficacy of the nicotine gum, nicotine patch and other NRTs, and (4) decreased load of carcinogens from other sources in the body (Box 2).

1. Facilitating oral nicotine as a form of NRT

The maximum tolerable dose of oral nicotine is around 4 mg, larger doses may cause gastric irritation [49]. Upon first pass through the liver, 70% of nicotine is metabolized to cotinine, reducing bioavailable nicotine to around 30% [49]. This high first-pass metabolism, coupled with the intestinal disturbances caused by high nicotine doses, restricts the use of oral nicotine in NRT because this method of delivery cannot provide sufficient nicotine to substitute for smoked nicotine, which typically delivers around 2 mg per cigarette [14].

Methoxsalen [50] (used in the treatment of psoriasis) and tranylcypromine [51] (a monoamine oxidase inhibitor used in the treatment of depression) are two clinically used drugs that inhibit CYP2A6. The effects of these drugs on plasma nicotine levels in nicotine-abstinent dependent smokers have been studied. 10 mg methoxsalen, 30 mg methoxsalen, 2.5 mg tranylcypromine or 10 mg tranylcypromine, when co-ingested with 4 mg oral nicotine, increased mean plasma nicotine concentrations by 72%, 83%, 43%, and 65%, respectively ($p<0.01$), above levels in subjects who ingested 4 mg oral nicotine and a placebo. These drugs also reduced subjects' self-rated current desire to smoke ($p<0.05$) [50,52]. This proof-of-concept study demonstrates that a new approach to an NRT is possible.

2. Exposure reduction

In a subsequent study, we examined whether the combination of CYP2A6 inhibition and oral nicotine could decrease smoking behaviour. We found that subjects who received methoxsalen and oral nicotine together smoked significantly less than those given placebo/placebo. This was reflected in a reduction in the smoking-related increase in breath carbon monoxide concentration of 50%, a 83% increase in latency to the second cigarette, a 24% decrease in the number of cigarettes smoked, a 24% decrease in grams of tobacco burned, and a 25% decrease in the total number of puffs taken (all $p<0.05$) [50]. These data suggest the use of a CYP2A6 inhibitor in combination with oral nicotine could represent a new treatment for nicotine dependence that is directed towards exposure reduction.

3. Enhancing the efficacy of nicotine gum, nicotine patches and other NRTs

Current forms of NRT (i.e. nicotine gum or patches) are the first-line choice for pharmacotherapy for smoking cessation; however, they are only moderately effective [53,54]. This is because these forms of nicotine uptake typically

provide only 50% of the mean plasma nicotine concentration that was obtained while smoking [55]. In addition, plasma concentrations of nicotine after a NRT are extremely variable and unpredictable because nicotine clearance is highly variable owing to variation in CYP2A6 activity. This high variation means that it is difficult to predict the effect of a fixed-dose NRT on any one patient. A treatment designed to inhibit CYP2A6 nicotine metabolism *in vivo* would be expected to improve the efficacy of existing NRT by increasing nicotine yield per unit dose (because of decreased nicotine clearance), by prolonging the duration of action of NRT, and by decreasing the variation in nicotine metabolism between and within individuals [43,50].

4. Decreasing body load of carcinogens

We hypothesized that CYP2A6 inhibition *in vivo* would result not only in decreased smoking behaviour but also in a decrease in the activation of procarcinogens to carcinogens. To examine this, smokers (N=11) were told to maintain their smoking levels by smoking the same number of cigarettes while receiving oral methoxalen 10 mg three times a day for 3 days. On day 3 of methoxsalen treatment, we observed a 29% increase in the ratio of plasma nicotine concentration/breath carbon monoxide concentration ($p=0.03$). Furthermore, significantly more NNK was metabolized to the inactive 4-(methylnitroamino)-1-(3-pyridyl)-1-butanol (NNAL)-glucuronide ($p<0.01$) relative to placebo. This demonstrated the re-routing of NNK from its CYP2A6-mediated mutagenic hydroxylation pathway to a non-mutagenic glucuronidation pathway, indicating a potential role for CYP2A6 inhibition in lowering the risk of lung cancer [56].

Models for proof-of-concept studies for new treatment of tobacco dependence

Several non-traditional study strategies can provide early evidence as to whether the treatment will be efficacious and an earlier understanding of how a novel treatment approach can be best deployed (Table 1; Box 3). First, some of these strategies arise from an awareness of the risk factors associated with smoking initiation and relapse (e.g. depressed mood, inadequate NRT and prior failure of treatment). Second, knowledge of these risk factors permits the employment of initial trials that use enrichment designs with fewer patients than traditional trials. Others design advantages arise from a more effective use of measures and markers of smoking. Cigarette smoking is a regulated frequent behaviour (around 400 puffs a day) for which excellent measures of patterns and extent of use are available (e.g. cigarettes per day, plasma cotinine concentrations, plasma nicotine concentrations, breath CO concentrations and video documentation of smoking pattern). Because the behaviour is frequent and relatively stable day-to-day, any short-term intervention administered in a properly blinded fashion that decreases indices of use over the short-term is very likely be efficacious in longer-term trials. Initial trials with the NRT gum used simple elegant rapid laboratory models to establish proof of concept [57]. Virtually all subsequent trials with NRTs or other treatment approaches have shown very high correlations between 48 h to 2 week outcomes and the outcomes of larger trials [22,58,59]. This argues strongly that the use of short-term proof-of-concept trials and a wider range of experimental designs than are used at present will have an important role in the development of medications for tobacco dependence. The studies of the manipulation of CYP2A6 are examples of brief cost-effective studies.

Finally, a broadening of our framework of treatment options might suggest further novel study strategies. Historically, cessation has been the focus of treatment for tobacco dependence. Cessation has been typically cast in a framework of abrupt cessation ('pick a quit date') augmented with adjunct use of a medication (e.g. NRT and bupropion). Clearly, stopping smoking is the desired public and individual health goal. In the context of a harmful behaviour that has continued for years and the fact that most smokers have tried (unsuccessfully) to stop or to cut down many times, however, there seems to be room to expand the repertoire of approaches that smokers have available as they achieve abstinence. For example, some smokers experience severe nicotine abstinence symptoms. For such smokers, NRT or other medications to ameliorate withdrawal could provide effective symptom relief when in situations in which smoking is not permitted (e.g. trans-Atlantic air travel) and constitute a needed treatment. Medications might also be used to initiate abstinence, prevent relapse (e.g. nicotine vaccine), reduce risk and exposure (e.g. CYP2A6 inhibition), or target special populations (e.g. antidepressants in smokers with depressed mood).

Box 3.

Strategies to shorter development of drugs for tobacco dependence

- Target risk factors associated with smoking or relapse; e.g. mood, triggers
- Use of an array of markers related to tobacco use and smoke exposure
- Target withdrawal, initiation of abstinence, relapse prevention and exposure reduction
- Employ short-term proof-of-concept studies as either clinical trials or experimental designs
- Consider genetic-based source of variation in response

Table 1. Models for tests of efficacy in treating tobacco dependence

Targeted outcome	Duration of proof-of-concept study	Model	Example of dependent variable
Acute withdrawal (e.g. blocker of withdrawal symptoms)	1 day	Abrupt cessation	Craving suppression Measures of tobacco dependence Changes in physical withdrawal symptoms
Initiate abstinence	Less than 2 weeks	Pre-treatment and monitor short-term outcome	Breath CO concentrations Plasma cotinine concentrations Cigarettes per day
Maintain abstinence	2–8 weeks	Smokers abstinent for longer than 1 week	Plasma cotinine concentrations Breath CO concentrations Self report
Reduction in use	A few days to few a weeks	Dependent smokers in an experimental setting or brief trial	Plasma nicotine concentrations Breath CO concentrations Latency time between cigarettes
Reduction in risk	Short term (weeks)	Decreased activation of pro-carcinogens	Urinary NNAL DNA adducts
	Long term (years)	Lung cancer	Ras oncogene conversion Incidence of lung cancer
Short-term cessation	2 weeks	Abrupt or step-care or tapered use leading to cessation	Breath CO concentrations Plasma cotinine concentrations Cigarettes per day
Relapse prevention	2–4 Weeks	Recently abstinent tobacco-dependent patients	Breath CO concentrations Plasma cotinine concentrations Cigarettes per day
Sub groups of at risk patients	2–4 Weeks	Patients with depressed mood Very heavy smokers Previous treatment failures	Breath CO concentrations Plasma cotinine concentrations Cigarettes per day

Several short-term proof-of-concept studies are possible for each of these drug treatments.

Importantly, when carefully designed and coupled with adequate measures of subjective reports on urge to smoke and measures of smoking topography, such studies can often lead to an optimal strategy for using the new treatment. Novel medications may not work optimally to provide a cessation outcome during the standard design 6–8 week abstinence trial. Adherence to such standard designs likely results in an underestimation of the therapeutic potential of new agents.

Conclusions

The health and economic costs of tobacco dependence worldwide are enormous. New treatment options for its prevention and treatment are in high demand. The clinical implications of CYP2A6 inhibition have provided new avenues of research that can help to facilitate oral nicotine as a form of NRT, promote exposure reduction, enhance the efficacy of the existing NRTs, and decrease body load of carcinogens. The traditional paradigm for developing medications for tobacco dependence needs to be re-examined and new approaches taken if we are to succeed in offering a more effective and broader range of treatments for smokers.

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