Selegiline Is a Mechanism-Based Inactivator of CYP2A6 Inhibiting Nicotine Metabolism in Humans and Mice

Eric C.K. Siu and Rachel F. Tyndale

Centre for Addiction and Mental Health and Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada

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ABSTRACT

Selegiline (L-deprenyl) is in clinical treatment trials as a potential smoking cessation drug. We investigated the effect of selegiline and its metabolites on nicotine metabolism. In mice, selegiline was a potent inhibitor of nicotine metabolism in hepatic microsomes and cDNA-expressed CYP2A5; the selegiline metabolites desmethylselegiline, L-methamphetamine, and L-amphetamine, also inhibited nicotine metabolism. Pretreatment with selegiline and desmethylselegiline increased inhibition (IC_{50}) in microsomes by 3.3- and 6.1-fold, respectively. In mice in vivo, selegiline increased AUC (90.7 ± 5.8 versus 57.4 ± 5.3 ng/h/ml, p < 0.05), decreased clearance (4.6 ± 0.4 versus 7.3 ± 0.3 ml/min, p < 0.05), and increased elimination half-life (12.5 ± 6.3 versus 6.8 ± 1.4 min, p < 0.05) of nicotine. In vitro, selegiline was a potent inhibitor of human nicotine metabolism in hepatic microsomes and cDNA-expressed CYP2A6; desmethylselegiline and L-amphetamine also inhibited nicotine metabolism. Selegiline preincubation increased inhibition in microsomes (3.7-fold) and CYP2A6 (14.8-fold); the K_{i} for CYP2A6 was 4.2 μM. Selegiline dose- and time-dependently inhibited nicotine metabolism by CYP2A6 (K_{i} = 15.6 ± 2.7 μM; k_{inact} = 0.34 ± 0.04 min^{-1}), and the inhibition was irreversible in the presence of NADPH, indicating that it is a mechanism-based inhibitor of CYP2A6. Thus, inhibition of mouse nicotine metabolism by selegiline was competitive in vitro and significantly increased plasma nicotine in vivo. In humans, where selegiline is both a competitive and mechanism-based inhibitor, it is likely to have even greater effects on in vivo nicotine metabolism. Our findings suggest that an additional potential mechanism of selegiline in smoking cessation is through inhibition of nicotine metabolism.

Nicotine is the primary psychoactive component in tobacco responsible for the addictive properties of cigarettes (Heningfield and Keenan, 1993). One action of nicotine is the binding to nicotinic receptors stimulating dopamine release in the nucleus accumbens, an area of the brain responsible for reward (Balfour, 2004). In the brain, dopamine is metabolized by monoamine oxidases (MAOs) A and B (Youdim et al., 2006), although MAO-B seems to be the major form (Fowler et al., 1987). The amount of MAO-B activity in the brain of smokers is ~40% lower compared with nonsmokers (Fowler et al., 1996), and this inhibition of MAO-B is reversed during long-term smoking abstinence (Gilbert et al., 2003), suggesting the presence of a MAO-B inhibitor in tobacco smoke (Khalil et al., 2000). These findings suggest that the reduced metabolism of dopamine, as mediated by the inhibition of MAO, may enhance the rewarding effects of nicotine during smoking (Lewis et al., 2007).

Selegiline is a selective and irreversible MAO-B inhibitor used in conjunction with 3,4-dihydroxy-L-phenylalanine to alleviate symptoms associated with Parkinson disease (Gerlach et al., 1996). Due to its ability to reduce dopamine metabolism, selegiline has been investigated as a potential therapy for smoking cessation. Several small-scale studies have shown that selegiline is effective in reducing withdrawal symptoms and increasing abstinence compared with placebo. For instance, in one study, 10 mg of oral selegiline decreased craving during abstinence and reduced smoking satisfaction during smoking (Houtschmiller et al., 2002). In another study, 5 mg b.i.d. oral selegiline increased the trial endpoint (8-week) 7-day point prevalent abstinence compared with placebo by 3-fold (George et al., 2003). In a third study that used a combination of oral selegiline and nicotine patch, selegiline plus nicotine patch doubled the 52-week...
continuous abstinence rate compared with nicotine patch alone, although the difference was not significant due to small subject numbers (Biberman et al., 2003). Other MAO inhibitors have also been investigated for their effects on smoking cessation, but due to poor efficacy ( moclobemide) or toxicity ( lazabemide), they are no longer studied (McRobbie et al., 2005).

Selegiline is metabolized to desmethylselegiline and L-methamphetamine, both of which can be further metabolized to L-amphetamine as well as other minor metabolites (Fig. 1) (Shin, 1997; Valoti et al., 2000); despite this, there is no evidence indicating that selegiline is addictive (Schneider et al., 1994). In humans, chronic treatment with selegiline reduces the metabolism of selegiline and its metabolites, suggesting that selegiline or its metabolites may inhibit or down-regulate its own metabolic enzymes (Laine et al., 2000). Selegiline belongs to the acetylene group of compounds that contain a carbon-carbon triple bond, which are known to be potent mechanism-based inhibitors (Correia and Ortiz de Montellano, 2005). Because the main human nicotine-metabolizing enzyme CYP2A6 seems to play a role in the metabolism of selegiline in vitro (Benetton et al., 2007), we examined whether selegiline and its metabolites (desmethylselegiline, L-methamphetamine, and L-amphetamine) (Shin, 1997) could inhibit nicotine metabolism in vitro in human and mouse hepatic microsomes, as well by both cDNA-expressed major human nicotine-metabolizing enzyme CYP2A6 and mouse CYP2A5 (Murphy et al., 2005; Siu and Tyndale, 2007). Genetically slow CYP2A6 metabolizers have a greater likelihood (1.75-fold) of quitting smoking (Gu et al., 2000), suggesting that if selegiline inhibits nicotine metabolism, this may be an additional mechanism through which it reduces smoking. In addition, selegiline could potentially be used to enhance the efficacies of current nicotine replacement therapies, or it could be combined with nicotine as an oral combination therapy with nicotine for smoking cessation.

**Materials and Methods**

**Animals.** Adult male DBA/2 mice, previously characterized in nicotine metabolism studies (Siu et al., 2007), were obtained from Charles River Laboratories Inc. (Saint-Constant, QC, Canada). Animals were housed in groups of three to four on a 12-h light cycle and had free access to food and water.

**Reagents.** (−)-Nicotine hydrogen tartrate, (−)-cotinine, selegiline, R-(−)-desmethylselegiline, thiamine hydrochloride, and 5-aminovaleric acid were purchased from Sigma-Aldrich (St. Louis, MO). L-Methamphetamine and L-amphetamine were purchased from BioShop Canada (Burlington, ON, Canada). Isopropyl β-d-thiogalactoside was purchased from MBI Fermentas (Burlington, ON, Canada). The monoclonal antibody to human CYP2A6 was purchased from BD Biosciences, (San Jose, CA). Horseradish peroxidase-conjugated anti-mouse secondary antibody and enhanced chemiluminescence were purchased from Pierce Biotechnology (Rockford, IL). Nitrocellulose membrane was purchased from Bio-Rad Laboratories Ltd. (Mississauga, ON, Canada). Autoradiographic film was purchased from Ulitdent Scientific (St. Laurent, QC, Canada).

**Membrane Preparations.** Microsomal membranes were prepared from mouse and human livers for in vitro nicotine metabolism assays as previously described (Siu et al., 2006) and stored at −80°C in 1.15% KCl. The human liver is from the previously characterized K-series liver bank (Messina et al., 1997) and was a generous gift from Dr. T. Inaba (University of Toronto, Toronto, ON, Canada). The cytosolic fractions were acquired during membrane preparation and were used as a source of aldehyde oxidase. All mouse livers and plasma samples were collected and frozen before 3:00 PM to minimize circadian effects on CYP2A5 expression. Membrane protein concentrations were determined with Bradford reagent according to the manufacturer’s protocol (Bio-Rad Laboratories Ltd.).

**Expression of CYP2A5.** The Cyp2a5 cDNA vector in Escherichia coli was a generous gift from Dr. Xinxin Ding (Wadsworth Center, New York State Department of Health, Albany, NY) and prepared as previously described with modifications (Gu et al., 1998). In brief, E. coli colonies from ampicillin plates were inoculated in 2 ml of Luria-Bertani broth with 100 μg/ml ampicillin and incubated overnight (no more than 16 h) at 37°C, shaking at 200 rpm. The culture was then diluted (1:100) in TB broth, with final concentrations of 100 μg/ml ampicillin, 1 mM thiamine, 0.5 ml of 5-aminovaleric acid, and 1 mM isopropyl β-d-thiogalactoside, and incubated for 48 h at 25°C, shaking at 150 rpm. After incubation the culture was centrifuged at 2800 g at 4°C for 20 min, and the pellet was resuspended in 1/20 culture volume of ice-cold TSE buffer (100 mM Tris, pH 7.4, 0.1 mM EDTA, 0.1 mM dithiothreitol, 1.15% KCl, 110 g/liter sucrose) and 1/20 culture volume of ice-cold water. Lysozyme was then added to a final concentration of 0.25 mg/ml, and this was shaken gently at 4°C for 1 h followed by centrifugation at 2800 g at 4°C for 20 min. After centrifugation the pellet was resuspended in 1/25 culture volume of ice-cold TE buffer. The resuspension was placed on ice and sonicated in 3 × 10-s bursts with a Branson Digital Sonifier (Model S-450D; Branson Ultrasonics, Markham, ON, Canada) at 30% output. The suspension was spun at 12,000 g at 4°C for 12 min, and the supernatant was resuspended in 180,000 g at 4°C for 60 min. The pellet containing the bacterial membrane fraction was resuspended in 1.15% KCl and stored at −80°C.

**Quantification by Immunoblotting of CYP2A5.** Immunoblotting was performed for CYP2A5 and the reference lymphoblastoid cDNA-expressed human CYP2A6 (BD Biosciences, San Jose, CA) essentially as described previously (Siu et al., 2006). To determine the linear ranges of detection of CYP2A5 and CYP2A6 to quantify the amount of CYP2A5 in the bacterial membrane, the bacterial membrane fraction and CYP2A6 were serially diluted from 0.25 to 2 μg of protein and from 0.06 to 1.5 pmol, respectively.

**In Vitro Nicotine C-Oxidation Assay.** The linear conditions for nicotine C-oxidation to cotinine (nicotine metabolism) in DBA2 mouse liver microsomes were described previously (Siu et al., 2006) (0.5 mg/ml protein; 15 min). In human liver microsomes, the linear conditions of nicotine metabolism were obtained under assay conditions of 0.5 mg/ml protein with an incubation time of 20 min. For CYP2A5, the linear conditions of nicotine metabolism were obtained...
under assay conditions of 60 pmol P450/ml and 60 pmol of reductase and cytochrome b5 (Invitrogen, Carlsbad, CA) with an incubation time of 15 min. For expressed CYP2A6 (containing P450 reductase and cytochrome b5) (BD Gentest, Woburn, MA), the linear conditions of nicotine metabolism were obtained under assay conditions of 20 pmol P450/ml with an incubation time of 15 min. All incubation mixtures contained 1 mM NADPH and 1 mM liver cytosol in 50 mM Tris-HCl buffer, pH 7.4, and were prepared at 37°C in a final volume of 0.5 ml. Unless specified, nicotine concentrations used were 10 (mouse liver microsomes), 60 (CYP2A5), 20 (human liver microsomes), and 100 (CYP2A6) μM. The reactions were stopped with a final concentration of 4% (v/v) Na2CO3. After incubation, 5-methyl-cotinine (70 μg) was added as the internal standard, and the samples were prepared and analyzed for nicotine and metabolites by HPLC as described previously (Siu et al., 2006). The limits of quantification were 5 ng/ml for nicotine and 12.5 ng/ml for cotinine.

In Vitro Inhibition of Nicotine Metabolism. In all in vitro inhibition experiments, assays were conducted as above with the addition of the inhibitors at the same time as nicotine. In experiments with the preincubation step, reactions containing increasing concentrations of the inhibitor (0–100 μM) were initiated by pre-warming the mixture for 2 min before the addition of NADPH and preincubation of 15 min at 37°C before addition of nicotine and incubation.

For the NADPH-dependent inactivation experiment, 0 or 100 μM of selegiline was added to the mixture in the presence or absence of NADPH. After preincubation, samples were loaded into the Micron YM-30 centrifuge membrane filters (Dyck and Davis, 2001) (Millipore, Etobicoke, ON, Canada) and centrifuged for at least 30 min at 4°C. Retentates (typically 40–50 μl) were added to fresh reaction mixtures containing nicotine, and the reactions were allowed to proceed.

In Vivo Inhibition of Nicotine Metabolism. Nicotine and selegiline were dissolved in physiological saline (0.9% sodium chloride) and adjusted to pH 7.4 for use in in vivo studies. All animals received injections i.p. with nicotine or nicotine plus selegiline (both at 1 mg/kg). The nicotine dose was chosen based on previous pharmacokinetic studies (Siu and Tyndale, 2007) and relevance in behavioral models for nicotine in mice (Marks et al., 1985). The selegiline dose was based on its relevance in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson disease (Fredriksson and Archer, 1995). Blood samples were drawn by cardiac puncture at various times after the injection. Immediately after collection, plasma was prepared by centrifugation at 3000g for 10 min and kept at −20°C until analysis. Total plasma nicotine levels (free and glucuronides) were measured after deconjugation by HPLC at ASPET Journals on October 17, 2017 jpet.aspetjournals.org Downloaded from

Results

Inhibition of Nicotine Metabolism by Selegiline and Metabolites. Selegiline and desmethylselegiline had the highest inhibitory activities on nicotine metabolism in mouse liver microsomes (MLMs), whereas l-methamphetamined and l-amphetamine had smaller effects (Fig. 2A). Selegiline also inhibited CYP2A5 to a great extent, whereas desmethylselegiline, l-methamphetamine, and l-amphetamine had similar inhibitory effects (Fig. 2B). In human liver microsomes (HLMs), selegiline and l-amphetamine seemed to show the greatest inhibitory activities on nicotine metabolism closely followed by desmethylselegiline (Fig. 2C), whereas l-methamphetamine did not inhibit cotinine formation (Fig. 2C). As with the three previous enzyme sources, selegiline caused the greatest inhibition on nicotine metabolism in CYP2A6 (Fig. 2D). Desmethylselegiline and l-amphetamine showed almost similar inhibition, whereas l-methamphetamine did not alter nicotine metabolism (Fig. 2D), as was seen with HLMs (Fig. 2C).

The above findings suggested that selegiline had the greatest inhibitory effect on CYP2A5-mediated nicotine metabolism in vitro in mice followed by the three metabolites of selegiline. Likewise, selegiline, desmethylselegiline, and l-amphetamine could inhibit CYP2A6-mediated human nicotine metabolism in vitro, whereas l-methamphetamine did not.

Effects of Selegiline and Desmethylselegiline Preincubation on Nicotine Metabolism in Mouse Liver Microsomes and CYP2A5. Because selegiline and desmethylselegiline contain a carbon-carbon triple bond found in mechanism-based inhibitors (MBIs), the effects of preincubation of these compounds on nicotine metabolism in MLMs and CYP2A5 were investigated. If pretreatment increased inhibition, this could indicate that the inhibitor is either acting as a MBI or is metabolized to a more potent inhibitor during the preincubation. Selegiline dose-dependently inhibited cotinine formation in both MLMs and CYP2A5; preincubation with selegiline decreased the IC50 in MLMs (3.3-fold) but not in CYP2A5 (Fig. 3, A and B). Desmethylselegiline also dose-dependently inhibited nicotine metabolism in MLMs, but its effect was much weaker in expressed CYP2A5 (Fig. 3, C and D). As with selegiline, preincubation with desmethylselegiline enhanced inhibition in MLMs (6.1-fold change in IC50) but not in CYP2A5 (Fig. 3, C and D). This indicated that selegiline and desmethylselegiline were competitive inhibitors, but not MBIs, of CYP2A5 in vitro, and other enzymes in MLMs can metabolize selegiline and desmethylselegiline to inhibitors of greater potency.

Effect of Selegiline on Nicotine Metabolism in vivo in DBA/2 Mice. To determine whether selegiline could also inhibit nicotine metabolism in vivo, we treated DBA/2 mice with selegiline and nicotine. Selegiline coadministration decreased the clearance of nicotine by ~40%, resulting in 58% greater AUC and almost doubling the elimination half-life (Fig. 4; Table 1). These results demonstrated that selegiline is an effective inhibitor of nicotine metabolism in vivo.
Preincubation of MLMs, but not CYP2A5, with selegiline increased inhibition of nicotine metabolism. MLMs (A and C) and CYP2A5 (B and D) were preincubated (+PRE) for 15 min with increasing concentrations (0, 0.3, 1, 3, 10, 30, and 100 µM) of selegiline (SEL), desmethylselegiline (DES), L-methamphetamine (L-MAMP), or L-amphetamine (L-AMP) before addition of nicotine and an additional incubation of 15 min. Activity remaining was calculated as a percentage of control (no inhibitor, same incubation conditions) within each treatment group. IC50 was determined as the concentration of inhibitor required to decrease nicotine metabolism by 50%. Each data point represented the average of two to five independent experiments. *, p < 0.05 compared with no preincubation.
Effects of Selegiline and Desmethylselegiline Pretreatment on Nicotine Metabolism in Human Liver Microsomes and CYP2A6. To further investigate the effect of selegiline and desmethylselegiline on nicotine metabolism in humans, HLMs and CYP2A6 were preincubated with the inhibitors. Selegiline dose-dependently inhibited cotinine formation in both HLMs and expressed CYP2A6 (Fig. 5, A and B). In contrast to the mouse enzymes, preincubation of both HLMs and CYP2A6 with selegiline decreased the IC$_{50}$ by 3.7- and 14.8-fold, respectively (Fig. 5, A and B). Desmethylselegiline also dose-dependently inhibited nicotine metabolism in HLMs (Fig. 5C) but seemed to be a weaker inhibitor of CYP2A6 (Fig. 5D). Preincubation with desmethylselegiline did not enhance inhibition in HLMs but may modestly increase inhibition in CYP2A6 (Fig. 5, C and D). The above findings suggested that selegiline is acting as a MBI of CYP2A6 or that selegiline could be metabolized by HLMs and CYP2A6 to metabolites that are even more potent inhibitors than selegiline. After preincubation, a $K_i$ of 4.2 $\mu$M for the competitive inhibition of CYP2A6 was observed (Fig. 6A).

TABLE 1
Pharmacokinetic parameters of plasma nicotine in mice treated i.p. with nicotine (1 mg/kg) or nicotine plus selegiline (1 mg/kg)
Results were derived using data (Fig. 3) from three to six animals at each time point.

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<th>−SEL</th>
<th>+SEL</th>
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<tr>
<td>AUC$_{0-40}$ (ng/h/ml)</td>
<td>57.4 ± 5.3</td>
<td>90.7 ± 5.8$^*$</td>
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<tr>
<td>$t_{1/2}$ (min)</td>
<td>6.6 ± 1.4</td>
<td>12.5 ± 6.3$^*$</td>
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<td>CL/F (ml/min)</td>
<td>7.3 ± 0.3</td>
<td>4.6 ± 0.4$^*$</td>
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$^*$ $p < 0.05$ compared to −SEL.

Fig. 4. Selegiline decreased nicotine metabolism in vivo in DBA/2 mice. DBA/2 mice received injections with nicotine (1 mg/kg i.p.) (−SEL) or with nicotine plus selegiline (1 mg/kg i.p.) (+SEL). After nicotine injection, plasma samples were collected at the indicated time points and analyzed for nicotine levels. Each time point represents mean (± S.D.) of three to six animals for each treatment; statistical comparisons are listed in Table 1.

Fig. 5. Preincubation of human hepatic microsomes and CYP2A6 with selegiline increased inhibition of nicotine metabolism. HLMs (A and C) and CYP2A6 (B and D) were preincubated (+PRE) for 15 min with increasing concentrations (0, 1, 3, 10, 30, and 100 $\mu$M) of selegiline (A and B) or desmethylselegiline (C and D) before addition of nicotine and an additional incubation of 15 or 20 min (HLMs). Samples were also incubated with the inhibitors and nicotine without pretreatment (−PRE). Activity remaining was calculated as a percentage of control (no inhibitor, same incubation conditions) within each treatment group. IC$_{50}$ was determined as the concentration of inhibitor required to decrease nicotine metabolism by 50%. Each data point represented the average of two to five independent experiments. $^*$, $p < 0.05$ compared with no preincubation.

Fig. 6. Time-, Concentration-, and NADPH-Dependent Irreversible Inhibition of CYP2A6-Mediated Nicotine Metabolism. In addition, to determine whether the inhibition of CYP2A6-mediated nicotine metabolism by selegiline is mechanism-based, we first carried out time- and concentration-dependent inactivation assays. Figure 6B indicates that the cotinine formation decreases with increasing preincubation time, and the decreases were dose-dependent. Using a double-reciprocal plot, the $K_i$ and $k_{inact}$ were estimated to be 15.6 ± 2.7 $\mu$M and 0.34 ± 0.04 min$^{-1}$, respectively (Fig. 6C).
To determine whether the decrease in nicotine metabolism was due to covalent modification of CYP2A6 by the metabolically activated selegiline, we used a centrifuge filtering system that would allow for the removal of the unbound inhibitors from the preincubation mixture (Dyck and Davis, 2001). In the absence of NADPH in the preincubation mixture with selegiline, filter removal of unbound selegiline did not lead to a significant decrease in enzyme activity compared with control (Fig. 6D), indicating a requirement for the cofactor NADPH. In contrast, in the presence of NADPH during preincubation, filter removal of unbound selegiline did not lead to significant recovery of cotinine formation activity, suggesting that CYP2A6 was inactivated by metabolic activation of selegiline. Similar findings were also seen with desmethyleselegiline (Fig. 6D). The above findings demonstrated that the inhibition of CYP2A6-mediated nicotine metabolism by selegiline is irreversible in the presence of NADPH and that selegiline is a MBI of CYP2A6.

**Discussion**

It is estimated that 20% of adults in the United States are current smokers (http://www.cdc.gov/nchs/fastats/smoking.htm). Previous studies demonstrated that selegiline was an effective aide in smoking cessation (Biberman et al., 2003; George et al., 2003). At least four clinical trials are currently underway to evaluate the effectiveness of selegiline as a potential therapy in smoking cessation (http://www.clinicaltrial.gov). Selegiline can prevent dopamine metabolism by irreversibly inhibiting MAO-B (Youdim, 1978). Selegiline also seemed to be able to inhibit its own metabolism in vivo (Laine et al., 2000), possibly via inhibition of CYP2A6. In the present study, we showed that nicotine metabolism in both mice and human liver microsomes and expressed enzymes could be inhibited by selegiline, desmethylselegiline, L-methamphetamine (only in mice), and L-amphetamine. In addition, we also demonstrated that selegiline inhibited nicotine metabolism in vivo in mice. More importantly, we showed that selegiline is a mechanism-based inhibitor of human CYP2A6.

In all in vitro systems tested in our study, selegiline (and desmethylselegiline in MLMs) inhibited nicotine metabolism with the greatest potency compared with its metabolites. In mice, a greater level of inhibition was seen in MLMs compared with the expressed CYP2A5. However, the enhanced inhibition of nicotine metabolism after selegiline preincubation in MLMs, not observed with CYP2A5, suggested that
selegiline is unlikely to be a MBI of CYP2A5. The greater inhibition in MLMs (compared with expressed CYP2A5; and with preincubation in MLMs) may be due to the formation of more potent inhibitory metabolite(s) of selegiline. None of the tested selegiline metabolites inhibited CYP2A5 with greater potency compared with selegiline. However, another metabolite that is produced in mice is selegiline-N-oxide (Levai et al., 2004). It is possible that in addition to the compounds tested here that selegiline-N-oxide, produced by other enzymes in MLMs, can also inhibit CYP2A5-mediated nicotine metabolism.

For both HLMs and CYP2A6, preincubation with selegiline led to an increase in inhibition potency. This enhancement in inhibition was more dramatic in CYP2A6 compared with HLMs, which might reflect the metabolism of selegiline in HLMs by other CYPs such as CYP2B6 and CYP2C8 (Benetton et al., 2007), thus reducing its availability to inhibit CYP2A6. More importantly, the inhibition of CYP2A6, in addition to being competitive, was probably mechanism-based. This was supported by the findings that in the time- and concentration-dependent inhibition study, the decrease in nicotine metabolism was enhanced with increasing inhibitor preincubation time and with increasing inhibitor concentration. Furthermore, the inhibition of CYP2A6 by selegiline was irreversible in the presence of NADPH, suggesting that selegiline was metabolically activated by CYP2A6, and the reactive intermediate formed a covalent linkage with the heme enzyme. The general mechanism of acetylene-mediated destruction of P450s appears to be the direct alkylation of the porphyrin (Correia and Ortiz de Montellano, 2005). However, the precise mechanism by which selegiline irreversibly inhibited CYP2A6 activity was not determined. The minor inhibition seen in the presence of selegiline without NADPH was probably due to residual inhibitor left in the retentate transferred to the fresh nicotine-containing reaction mixture.

Because desmethylselegiline also contains the carbon-carbon triple bond and irreversibly inhibited MAO-B and decreased its activity by up to 65% in vivo (Heinonen et al., 1997), we also characterized its effects on nicotine metabolism in both species. In mice in the absence of preincubation, desmethylselegiline inhibited nicotine metabolism in MLMs to the same extent as selegiline. The greater inhibition by desmethylselegiline in MLMs (compared with CYP2A5) and the potentiation of inhibition with preincubation are probably due to the production of more potent metabolite(s) of desmethylselegiline by other enzymes in MLMs. The only known metabolites of desmethylselegiline are L-amphetamine and its hydroxylated products (Shin, 1997); however, L-amphetamine had weaker inhibitory activities. Thus, the specific desmethylselegiline metabolite (e.g., potentially N-oxides) that may be contributing to the inhibition in MLMs, but not in CYP2A5, is unknown.

It is interesting to note that in HLMs, preincubation with desmethylselegiline did not increase the inhibition potency, but it appeared to show minor effects on CYP2A6. One possibility is that desmethylselegiline had weaker affinity for CYP2A6 compared with other enzymes present in HLMs (i.e., inhibiting or metabolized by other enzymes) and thus was less available to inhibit nicotine metabolism by CYP2A6. The inhibition of CYP2A6 by desmethylselegiline could potentially be mechanism-based because in the presence of NADPH, the inhibition of nicotine metabolism was irreversible.

In our study, L-methamphetamine inhibited nicotine metabolism in mice but not in humans. L-amphetamine inhibited nicotine metabolism almost to the same extent as selegiline in CYP2A5 and HLMs but had the weakest effect in CYP2A6. Again, it is possible that L-amphetamine was metabolized to more potent inhibitors of nicotine metabolism by other enzymes in HLMs. The effects of the D-isomers of methylamphetamine and amphetamine on CYP2A5 or CYP2A6 activities are unknown; however, one study found that racemic (DL-)amphetamine was a weak inhibitor of CYP2A6 and a much weaker inhibitor of CYP2A5 (Rahnasto et al., 2003).

We and others have previously demonstrated that nicotine, at relevant pharmacological concentrations, is metabolized essentially exclusively by CYP2A5 in mice (Murphy et al., 2005; Siu and Tyndale, 2007). We have also shown that s.c. administration of the CYP2A5/6 inhibitor methoxsalen significantly inhibited nicotine metabolism when nicotine was given s.c., and this increase in nicotine plasma levels subsequently increased the pharmacological actions of nicotine (Damaj et al., 2007), demonstrating the value of mice in studying nicotine metabolism and pharmacology. Because selegiline inhibited CYP2A5-mediated nicotine metabolism in MLMs and CYP2A5, we determined its effect on nicotine clearance in mice in vivo. We treated mice with nicotine and selegiline i.p. because this route mimics oral delivery of both drugs in animals, the route of choice for a novel smoking cessation product. Administration of selegiline together with nicotine caused almost a 40% decrease in clearance of nicotine and doubled its elimination half-life. These data suggested that selegiline and its metabolites can act as competitive inhibitors of nicotine in vivo even though selegiline is not a MBI of CYP2A5. It is likely that in humans, selegiline can decrease the first-pass metabolism and elimination half-life of nicotine via mechanism-based inactivation as well as competitive inhibition of CYP2A6. Genetically slow CYP2A6 metabolizers have an increased likelihood of quitting smoking (Gu et al., 2000), suggesting that inhibition of CYP2A6 by selegiline may contribute to selegiline’s ability as a smoking cessation therapeutic agent.

Our findings suggest that oral selegiline may also be combined with oral nicotine to create an orally bioavailable and clinically effective form of nicotine due to the high hepatic extraction of selegiline (Heinonen et al., 1994) (suggesting that selegiline will be able to rapidly inhibit CYP2A6 before reaching the systemic circulation) and the relatively smaller Kᵢ and Kᵢₑ of selegiline toward nicotine by CYP2A6 (4.2 and 15.6 μM, respectively) compared with the Km for nicotine (~65 μM) (Messina et al., 1997). At this time, there is no pill of nicotine replacement therapy because the higher doses required to overcome first-pass metabolism may cause nausea and diarrhea due to gastrointestinal irritation (Brown et al., 1991). Oral administration of even low doses (4 mg) of nicotine to those genetically deficient in CYP2A6 increased nicotine AUC by over 3-fold (Xu et al., 2002), suggesting that inhibition of nicotine metabolism can make nicotine orally bioavailable. We have also shown that the combination of the CYP2A6 inhibitor methoxsalen and nicotine significantly increased the mean plasma level of nicotine in humans as well as decreased the number of cigarettes smoked, reduced craving, the number of puffs, the inhalation intensity, and breath carbon monoxide levels, a biomarker of smoke inhalation (Sellers et al., 2000). Furthermore, inhibi-
tion of CYP2A6 by methoxsalen also rerouted the tobacco-specific procarcinogen (4-methylnitrosamine)-1-(3-pyridyl)-1-butanone to 4-(methylthiosemicarbazono)-1-(3-pyridyl)-1-butanone and glucuronidation pathways (Sellers et al., 2003), indicating the additional potential benefit for harm reduction.

In this study, we have shown that selegiline is a mechanism-based inhibitor of human CYP2A6, the major enzyme responsible for nicotine metabolism. In mice, where selegiline and its metabolites are competitive inhibitors, selegiline substantially reduced nicotine metabolism in vivo, resulting in prolonged elevated nicotine plasma levels. These findings suggest that in humans, where selegiline is a competitive and MBI of CYP2A6-mediated nicotine metabolism, selegiline can potentially be an effective inhibitor of nicotine metabolism.

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References


Address correspondence to: Dr. Rachel F. Tyndale, Department of Pharmacology, University of Toronto, 1 King’s College Circle, Room 4326, Toronto, Canada M5S 1A8. E-mail: r.tyndale@utoronto.ca