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Alcoholism, smoking and cyp-mediated drug metabolism

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Alcoholism, Smoking and CYP-mediated Drug Metabolism

Liver
Genetic variation
Drug Dependence and Consumption

Brain
CYP regulation and expression
Drug action, toxicity, mutagenicity
Outline of the talk

CYP2A6: nicotine metabolizing (major liver)
- Genetic variation
- Down regulation by nicotine (↓ 30%)

CYP2B6: nicotine metabolizing (very minor liver, ? brain)
- Genetic variation
- Up regulation by nicotine and ethanol (organ specific)

CYP2E1: ethanol metabolizing (minor liver, ? brain)
- Genetic variation
- Up regulation by nicotine and ethanol (liver and brain)
Acknowledgements

Collaborators
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- Neal Benowitz
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- Deborah Mash

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- PNAT and NIDA: DA06889
- Centre for Addiction and Mental Health Nicogen Research Inc

Dec 1942
Genetically Reduced CYP2A6 increases nicotine plasma levels

Nicotine 4 mg base, oral   Japanese subjects

- **Inactive** $*4/*4$
- **Decreased** $*1/*4$
- **Active** $*1/*1$

Nicotine plasma (ng/ml) vs Time (hours)

- **Slow Metabolizers**
- **Normal Metabolizers**
Does slow nicotine inactivation reduce smoking?

Rationale:
- Dependent smokers adjust their smoking behavior to maintain nicotine levels
- Amount smoked ≈ 85% genetic
- Risk for smoking and stopping smoking 50-70 % genetic

Slow Nicotine Inactivation may:
- reduce amount smoked/day – less behaviour
- reduce positive vs aversive effects
- reduce withdrawal and tolerance and neuroadaptation

Hypotheses:
Slow nicotine metabolizers
- will smoke fewer cigarettes per day
- will be less likely to be regular, dependent smokers
- will smoke for less time, quit sooner
- respond better to NRTs
**Slow metabolism decreases smoking**

(#{ of cigarettes smoked and breath CO})

Rao et al., Mol Pharm 2000

<table>
<thead>
<tr>
<th>Plasma NIC (ng/ml)</th>
<th>Cigarettes/Day</th>
<th>Carbon Monoxide ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Significant</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.005</td>
</tr>
</tbody>
</table>

**Normal Metabolizers**

*1/*1 n=277

**Slow Metabolizers**

*1/*2 & *1/*4 n=14
Ethnic differences in daily smoking: Effect of CYP2A6

- African Americans (N=120)
- Caucasians (N=361)
- Japanese (N=1705)

- Normal
- Slow
- Inactive

Goodz NTR 2002
Schoedel Pharmacogenetics 2004
Fujieda Carcinogenesis 2004
CYP2A6 slow metabolizers: Decreased risk for being a current smoker

Caucasians “Slow” Nicotine metabolizers:
- 1 inactive (*2, *4) or
- 2 low activity (*9, 12) alleles

- less frequent in smokers vs non-smokers:
  Odds Ratio = 0.52
  (95% CI: 0.30-0.92)

Age, sex, education, drugs

- indicates slow nicotine inactivators are less likely to be current smokers

Schoedel et al Pharmacogenetics, 14: 615, 2004
CYP2A6 slow metabolism reduces duration of smoking

Caucasians “Slow” Nicotine metabolizers:

- smoke for a shorter duration
- slow nicotine inactivators are less likely to be current smokers as duration of smoking increases

<table>
<thead>
<tr>
<th>Years of Smoking</th>
<th>NonS</th>
<th>&lt;10</th>
<th>11-20</th>
<th>21-30</th>
<th>&gt;31</th>
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<tr>
<td></td>
<td></td>
<td>5</td>
<td>6.9</td>
<td>6.0</td>
<td>5.6</td>
</tr>
<tr>
<td>0</td>
<td>12.5</td>
<td>9.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Schoedel et al Pharmacogenetics, 14: 615, 2004
Pharmacogenetics of Treatment

Current treatment: low efficacy

- Genetic variation in response
- Tailor medication to genetic make-up
- Discover novel targets for drug development
Genetically Slow Metabolizers have higher nicotine on the patch (treatment seeking population)

Nicotine Patch Usage
(# days worn)

Plasma Nicotine
(ng/mL)

SD

Malaiyandi et al, Mol Psychiatry 2006
Genetically Slow Metabolizers have lower usage & equal nicotine using the spray (treatment seeking population)

Nicotine Spray Usage (doses/day) vs. Plasma Nicotine (ng/mL) with SD.

*p<0.02*

Malaiyandi et al, Mol Psychiatry 2006
Smoking Cessation Rates by Nicotine Metabolite Ratio and Treatment

Lerman et al, CPT 2006

Transdermal Patch  n=240  Nasal Spray  n=240

End of treatment
6-month

% Quit

1st Qrtl 2nd Qrtl 3rd Qrtl 4th Qrtl 1st Qrtl 2nd Qrtl 3rd Qrtl 4th Qrtl

Effect of ratio in TP group (p=.005); Wald Test for interaction of ratio by treatment (p=.04)
Male African Green Monkey (Cercopithecus aethiops)  

Methods

- Male Monkeys (n=6 per group) treated for 22 days with:
  - saline (s.c., BID)
  - nicotine (s.c., 0.3 mg/kg, BID)

- Smoker ~ 0.5 mg/kg nicotine /day
- Monkey 0.6 mg/kg nicotine/day (slightly faster metabolism)
In vitro Nicotine metabolism is decreased by in vivo treatment

Schoedel et al., Mol Pharm 2003

**Velocity of Nicotine Metabolism**

(ng cotinine /mg protein/min)(-SD)

- **P<0.05**
- ↓ ~40%
In vivo Nicotine decreases the amount of hepatic CYP2A6 protein and mRNA (Western & Northern blotting, N=6/group)

- **Amount of CYP2A protein (± SD)**
  - Saline: ~250 units
  - Nicotine: ~60% decrease (~100 units)

- **Amount of CYP2A mRNA (± SD)**
  - Saline: ~1.2 units
  - Nicotine: ~35% decrease (~0.8 units)

**Statistical Significance:**
- P<0.05
- HL AGM
Genetic variation:

Decreased CYP2A6 nicotine metabolism:
-↓ Number of Cigarettes/day
-↓ Risk to be an adult smoker
-↓ Duration Tobacco Dependence
-↑ Success of Quitting
-↑ Success on Nicotine Patch

Regulation:

Alcohol/alcoholism
- ➢ no effect on activity

Nicotine/Smoking
- ↓ rates of nicotine metabolism

Rao et al., 2000; Xu et al., 2002; Schoedel et al. 2003 & 2004; Maliyandi et al., 2006; Lerman et al., 2006
Human CYP2B6 and Rat CYP2B1

Alters Drug and Metabolite concentrations
- Inactivate Drugs: Nicotine (central metabolic tolerance?)
- Activate Drugs: Bupropion (altered efficacy?)
- Endogenous Substrates: Testosterone

Genetically variable and Inducible
- Highly genetically polymorphic
- Nicotine and Ethanol induce (organ/species specific)

Cause Toxicity
- Activate drugs to toxic metabolites: Cocaine, PCP, MDMA
- Produce oxygen radicals and lipid peroxidation

Mutagenicity and Genotoxicity
- Activate tobacco-smoke procacinogens (i.e. NNK, NDMA, NMA)
Nicotine and Ethanol
CYP2B1 and CYP2E1

Alcoholics versus non-alcoholics:
- 95% smokers vs 25%
- smoke more (also social drinkers)
- are more tolerant to nicotine
- have greater tobacco withdrawal

Smokers versus non-smokers:
- drink 2 x more ethanol
- have faster ethanol elimination

Alcohol and Smoking
- Common genetic susceptibility (e.g. 10q24.3)
- Nicotine increases ethanol self-administration in rats and vice versa
- Synergistic increase in disease susceptibility in alcohol abusers who smoke

Mechanisms:
- functional tolerance (receptor adaptation)
- metabolic tolerance (enzyme induction)

Nicotine and ethanol selectively regulate CYP enzymes in the brain.
CYP in Brain: Unique localizations and regulation

- Alter drug concentrations near receptors
- Alter endogenous functions
- Cause toxicity or mutagenicity

Exquisitely sensitive to drugs, complex patterns of regulation (tissue and cell-type specific)

Functional (ex vivo, in vitro)

Regulated (e.g. organ, region, cell, membrane)

Highly localized (e.g., by region, cell type, membrane)
CYP2B6 is found in both neurons and astrocytes in human brain.

A. Astrocytes around a cerebral blood vessel in Frontal Cortex layer I

B. Neurons in Frontal Cortex layer II

C. Pyramidal neurons in Frontal Cortex layer III

D. Purkinje cells in Cerebellum

Miksys et al Neuropharmacology 2003
Human CYP2B6 in Human Frontal Cortex layer I

Miksys et al. Neuropharmacology 2003

Cerebral Blood Vessel

Astrocytes

CYP2B6: texas red

GFAP: FITC

Costained: yellow

Astrocytic processes stained for CYP2B6 & GFAP

100 μm
CYP2B6 is higher in Cerebellar Purkinje cells of *Smokers*

Miksys et al Neuropharm 2003

Cont. M P GL

Non-Smoker

Smoker

M – molecular layer
P – Purkinje cell layer
GL – granule cell layer
Bar: 40 µm
Alcoholics and Smokers have higher CYP2B6 in specific brain regions

- Are the higher levels of CYP2B6 in brain due specifically to alcohol and nicotine?

Models
- Human neuroblastoma cell lines
- Rats treated in vivo
- Monkeys treated in vivo
Nicotine Induction of Rat CYP2B1 is Brain Region Specific
(1mg base/kg/day x 7 days)

OB - olfactory bulb, ST - striatum, FC - frontal cortex, OT - olfactory tubercle, CB - cerebellum, HC - hippocampus, BS - brain stem
Nicotine induces CYP2B in Frontal Cortex neurons (1 mg/kg s.c. x 7 days)

4 hr post injection

24 hr post injection 7 day post injection

Bar: 100 μm
Nicotine (1 mg/kg x 7 days) induces CYP2B in Piriform Cortex neurons (Bregma 3.2) in layer II (C, arrow)

CYP2B1 increases in Brainstem with Nicotine Dose (both protein and mRNA)
Male African Green Monkey (Cercopithecus aethiops)  

Methods

- Male Monkeys (n=6 per group) treated for 22 days with:
  - saline (s.c., BID)
  - nicotine (s.c., 0.3 mg/kg, BID)

- Smoker ~ 0.5 mg/kg nicotine /day
- Monkey 0.6 mg/kg nicotine/day (slightly faster metabolism)
Nicotine does not induce hepatic CYP2B6 in monkey liver (same as rats and smokers)

Lee et al., Neuropharm 2006
Nicotine increases CYP2B6 in monkey brain (frontal cortex) (Lee et al., Neuropharm 2006)

Frontal Cortex

1.9-fold increase in CYP2B6 levels in nicotine treated monkeys

CYP2B6 units

p=0.002

Control

Nicotine
<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Control</th>
<th>Nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortical pyramidal cells</td>
<td>★ ★ ★ ★</td>
<td>★ ★ ★ ★</td>
</tr>
<tr>
<td>Dentate gyrus granular layer</td>
<td>★ ★</td>
<td>★ ★ ★</td>
</tr>
<tr>
<td>Caudate neuronal cells</td>
<td>★ ★</td>
<td>★ ★ ★ ★</td>
</tr>
<tr>
<td>Thalamic ventral lateral nucleus</td>
<td>★ ★</td>
<td>★ ★ ★ ★</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1 region</td>
<td>★ ★</td>
<td>★ ★ ★ ★</td>
</tr>
<tr>
<td>CA2 region</td>
<td>★ ★</td>
<td>★ ★ ★ ★</td>
</tr>
<tr>
<td>CA3 region</td>
<td>★ ★</td>
<td>★ ★ ★ ★</td>
</tr>
<tr>
<td>subiculum</td>
<td>★ ★</td>
<td>★ ★ ★ ★</td>
</tr>
</tbody>
</table>

All other brain regions examined did not show induction by nicotine.
Chronic Ethanol (gavage x 7 days) Increases CYP2B1 protein in rat liver

Schoedel et al., Biochem Pharm 2001
Chronic ethanol (i.p. x 7 days) Increases CYP2B1 mRNA in rat liver

Schoedel et al., Biochem Pharm 2001
Chronic ethanol (i.p. x 7 days) increases Nicotine metabolism to Cotinine in rat liver

Approximately 70% of Nicotine metabolism is by RAT CYP2B1/2 in both EtOH induced and uninduced states

Schoedel et al., Biochem Pharm 2001
Chronic Ethanol Does Not Alter CYP2B1/2 Protein in Rat Brain (Immunoblotting)
Nicotine Metabolizing CYP2B1

Genetics:
CYP2B6*6 Associated with Smoking cessation

Regulation:

Nicotine
- Liver: No effect in rat or monkey
- Brain: Increases in rat and monkey brain
  Liver and Brain, consistent with human data

Ethanol
- Liver: Increase rat liver CYP2B1 protein, activity and mRNA
- Brain: No effect on rat brain CYP2B1
  Liver consistent with human data
  Brain not consistent (species/primate or inducer?)

Miksys et al., Biochem Pharm 2000; Schoedel et al., Biochem Pharm 2001; Miksys et al., Neuropharm 2003; Schoedel et al., Mol Pharm 2003; Schoedel et al., BBA 2003; Lee et al Neuropharmacology 2006
CYP2E1

Drug Metabolism
- Inactivates ethanol (metabolic tolerance)
- Endogenous role - gluconeogenesis

Toxicity
- Activates drugs (halothane, acetaminophen)
- Activates procarcinogens (cigarette smoke nitrosamines)
- Generates reactive oxygen species (lipoperoxidation)

Inducibility
- Ethanol (metabolic tolerance)
- **Cigarette smoke**, increases CYP2E1 activity
- **Smokers** have faster **alcohol** elimination rates (tolerance/ cross-tolerance / alcohol-related disease ?)
  
  Lieber 1999; Kunitoh et al., 1997; Albano et al 1999; LeClerq et al 2000
Dramatic inter-ethnic variation in CYP2E1*1D frequencies

Howard et al., Pharmacogenetics 2003
Smokers versus non-smokers:
- 95% of alcoholics are smokers
- smokers drink more alcohol
- higher alcohol elimination rates

Nicotine
- ↑ self-administration of alcohol in rodents
- ↑ rate of dependence on alcohol in rodents

Mechanisms:
- functional tolerance (e.g. nicotinic acetylcholine receptors)
- metabolic tolerance (increased alcohol metabolism)

Tobacco smoke ↑ 2E1 activity in rodents & humans

In vivo chlorzoxazone clearance (CYP2E1) is increased following smoking in humans

Benowitz 2003

Increased clearance mediated by CYP2E1?
CYP2E1 in Human Cerebellum
(Protein: Immunohistochemistry)

Non-alcoholic non-smoker

Alcoholic non-smoker

Alcoholic Smoker

Molecular Layer

Purkinje Cells

Granular Layer

↑ CYP2E1 in purkinje cells and possibly granular layer

Howard et al., Br J Pharmacol 2003
CYP2E1 in Human Frontal Cortex
(Protein: Immunohistochemistry)

No Antibody  Non-alcoholic non-smoker  Alcoholic non-smoker  Alcoholic smoker

Cortical layers  300 μM  20 μM  300 μM  20 μM  300 μM  20 μM

White matter  50 μM  50 μM  50 μM

Howard et al., Br J Pharmacol 2003
Nicotine Induces CYP2E1 in Human Neuronal Cell Line (IMR-32)

Howard et al., Br J Pharmacol 2003
Dose-dependent increase in Rat hepatic CYP2E1 by Ethanol and Nicotine

Howard et al., JPET 2001

**Ethanol**

CYP2E1 protein

<table>
<thead>
<tr>
<th></th>
<th>0.3 g/kg</th>
<th>1.0 g/kg</th>
<th>3.0 g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td><img src="saline_0.3gkg.png" alt="Image" /></td>
<td><img src="saline_1.0gkg.png" alt="Image" /></td>
<td><img src="saline_3.0gkg.png" alt="Image" /></td>
</tr>
<tr>
<td>p&lt;0.05</td>
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<td><img src="bar_1.0gkg.png" alt="Image" /></td>
<td><img src="bar_3.0gkg.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Nicotine**

CYP2E1 protein

<table>
<thead>
<tr>
<th></th>
<th>0.1 mg/kg</th>
<th>0.3 mg/kg</th>
<th>1.0 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td><img src="saline_0.1mgkg.png" alt="Image" /></td>
<td><img src="saline_0.3mgkg.png" alt="Image" /></td>
<td><img src="saline_1.0mgkg.png" alt="Image" /></td>
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<td><img src="bar_0.3mgkg.png" alt="Image" /></td>
<td><img src="bar_1.0mgkg.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Centrilobular Induction of rat CYP2E1 by Ethanol and Nicotine

Howard et al., JPET 2001
Ethanol and Nicotine Increase rat CYP2E1 Catalytic Activity

Vmax for 6OH chlorzoxazone production is increased, affinity (Km) unaltered

Howard et al., JPET 2001
Rat hepatic CYP2E1 is induced after 7 days by **Nicotine** with a very low ED$_{50}$ (0.01 mg/kg s.c.)

Micu et al., JPET 2003

**Nic/Sal density units (mean + SD)**

ED$_{50}$ ~ 0.01 mg/kg

Tx: 0.001 - 1.0 mg/kg Nic s.c.
ST: 4 hrs post tx

Nicotine dose in mg/kg s.c.
Immunocytochemical data suggest basal & induced CYP2E1 predominantly glial cell expression.
Male African Green Monkey (Cercopithecus aethiops)

Methods

- Male Monkeys (n=6 per group) treated for 22 days with:
  - saline (s.c., BID)
  - nicotine (s.c., 0.3 mg/kg, BID)
- Smoker ~ 0.5 mg/kg nicotine /day
- Monkey 0.6 mg/kg nicotine/day (slightly faster metabolism)
CYP2E1 is induced by Nicotine in Monkey Frontal Cortex

Joshi & Tyndale, Neuropharmacology, 2006

Relative CYP2E1 levels

Saline

Nicotine

1.45 fold

p < 0.05

Bar 100 μm

No Primary
Ethanol Metabolizing CYP2E1

Genetics
CYP2E1*1D (induction variant) associated with alcohol & tobacco dependence

Regulation
Nicotine

↑Liver: Increases hepatic CYP2E1 ED$_{50}$ = 0.01 mg/kg (not mRNA)
↑Brain: Increases CNS CYP2E1 (not mRNA)

Consistent with human data

Ethanol

↑Liver: increases rat hepatic CYP2E1 (not mRNA)
↑Brain: increases CNS CYP2E1 (not mRNA)

Consistent with human data

Howard et al, JPET 2001; Howard et al., Br J Pharm 2003; Micu et al., JPET 2003; Howard et al., Pharmacogenetics 2003; Schoedel & Tyndale, BBA 2003; Joshi & Tyndale, Neuropharm 2006; Joshi & Tyndale, DMD 2006
Know only a little about the function and regulation of brain CYP enzymes
**Implications**

Interindivdual differences in drug metabolism can alter:
- Drug abuse liability
- Risk for drug dependence
- Amount of a drug consumed
- Ability to quit
- Toxicity

Enzyme Induction (e.g. by nicotine or ethanol) can up and down regulate enzymes altering:
- Nicotine and ethanol metabolism (and other drugs)
- Pharmacological actions: drug effect, drug taking
- Metabolic tolerance/ Metabolic Cross-Tolerance
- Toxicity of enzymatic pathway – contribute to disease

Effects are organ specific
And Thanks to
The Gang

Ewa Hoffman
Sharon Miksys