Inhibition of Aldehyde Dehydrogenase-2 (ALDH-2) Suppresses Nicotine Self-Administration in Rats

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Abstract
Introduction. Aldehyde dehydrogenase-2 (ALDH-2) inhibitors have been shown to reduce cocaine and alcohol intake in rats. The mechanism of action appears to be due to inhibition of drug-induced dopamine (DA) production in the ventral tegmental area (VTA) and DA release in the nucleus accumbens. The purpose of this study was to explore the potential of a selective ALDH-2 inhibitor to reduce self-administration of nicotine. Materials and methods. Adult male rats were trained to self-administer nicotine intravenously. After acquiring a stable baseline for nicotine intake, rats were given one of the three oral gavage doses (5, 10 or 30 mg eq/kg, calculated based on parent drug) of the prodrug GS-6637 of an ALDH-2 inhibitor or vehicle one hour before a nicotine self-administration session. Results. Our data showed that acute administration of GS-6637 at 10 mg eq/kg and 30 mg eq/kg significantly reduced nicotine self-administration. Similarly, subchronic administration of GS-6637 for seven days significantly reduced nicotine self-administration at 10 mg eq/kg and 30 mg eq/kg without inducing tolerance. In order to compare GS-6637 with varenicline, rats were given single doses of varenicline at 1.6, 3.2, and 6.4 mg/kg. Consistent with previous reports, significant inhibitions of nicotine self-administration was observed at the 3.2 mg/kg and 6.4 mg/kg doses but less than observed with GS-6637. Discussion and conclusions. Our data suggest that selective ALDH-2 inhibition appears to have therapeutic potentials as novel therapy for smoking cessation.

Keywords nicotine addiction; treatment; smoking cessation; dopamine; inhibition; aldehyde dehydrogenase-2; self-administration

1. Introduction
Nicotine addiction arising from smoking tobacco is a major health problem. Approximately 1.1 billion people worldwide smoke tobacco. Although the prevalence of smoking in North America has been decreasing and currently is about 23%, smoking is much more prevalent in developing countries and continuing to rise. The health consequences of smoking and costs to the society are enormous. Tobacco smoking is the most common cause of cancer-related deaths and the leading cause of heart disease, emphysema, and bronchitis [1]. Among thousands of artificial and natural constituents in tobacco smoke, nicotine is the main psychoactive constituent that underlies smoking addiction. Nicotine exerts its reinforcing effects by binding to neuronal nicotinic acetylcholine receptors (nAChRs), leading to enhanced release of dopamine (DA) and other neurotransmitters involved in reward and pleasure [2].

Among the brain monoaminergic neurotransmitter systems, the dopaminergic system has been most clearly implicated in reinforcing properties of addictive drugs including nicotine [3,4,5,6,7]. A wide range of animal studies as well as in vivo imaging have demonstrated that DA plays a major role in reinforcing properties of drugs of abuse including nicotine [8]. Nicotine is not the only psychoactive compound in cigarette smoke. In addition to nicotine itself, other natural and added constituents of cigarette smoke might also contribute to smoking addiction. For example, condensation products of acetaldehyde with biogenic amines in cigarette smoke inhibit the activity of the enzyme monoamine oxidase (MAO), which leads to a decrease in DA metabolism and consequently an increase in DA concentration [9]. Mesolimbic DA neurons express high-affinity nAChRs both on their cell bodies and terminals [10]. Moreover, presynaptic nAChRs on midbrain DA neurons project from the ventral tegmental area (VTA) to the nucleus accumbens and prefrontal cortex. These presynaptic nAChRs evoke DA release when they are activated by nicotine (tobacco smoking) [2] or alcohol administration [11,12,13]. It has been reported that acute nicotine administration enhances striatal and limbic DA turnover and metabolism [14]. Nicotine also elevates extracellular DA in the striatum and in the nucleus accumbens of rats [7,15,16]. Additionally, the demonstration that nicotine can block DA reuptake [17] strengthens the hypothesis that DA mechanisms may be important in nicotine reinforcement.

Current available therapies to reduce tobacco use include nicotine replacement, bupropion, and varenicline (Chantix; Pfizer, KS, USA) [18]. However, they have limited success
rates in the large majority of people attempting to quit or trying to maintain long-term abstinence [19]. Recently, it
has been shown that inhibition of aldehyde dehydrogenase-2 (ALDH-2) can suppress cocaine intake [20] and alcohol
intake in rats [21]. The mechanism of action of ALDH-2 inhibition has been shown to involve suppression of DA pro-
duction [21]. Furthermore, recently we showed that another selective mitochondrial ALDH-2 inhibitor, GS-455534 (pre-
viously known as CVT-10216), at low doses significantly reduces alcohol intake in alcohol preferring rats [22]. These
findings suggest that ALDH-2 inhibitors might also reduce nicotine intake.

The aims of the current studies were (1) to determine the acute oral dose-response of GS-6637, a novel selective
ALDH-2 inhibitor, on nicotine self-administration, (2) to learn whether tolerance develops after repeated oral
administration of GS-6637, and (3) to compare GS-6637 efficacy to varenicline. It is hypothesized that
inhibition of ALDH-2 by GS-6637 will reduce nicotine self-administration.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley albino rats (225–250 g at arrival) were singly housed in approved standard laboratory
conditions at the Duke University vivarium facility next to the testing room to minimize any stress induced by
transporting the rats. The day-night cycle was reversed so that rats were in their active phase during behavioral testing.
All rats had ad lib access to water and were fed the same type of rat chow once daily throughout the study to keep
them at approximately 85% ad lib weight with food amounts adjusted from 8 g to 16 g per day as they grew to provide
a lean healthy growth curve. The procedures used in this study were approved by the Duke University Animal Care
and Use Committee and conform to the Animal Care Guide.

2.2. Nicotine self-administration

Three sets of experiments were carried out. First, we established a dose-response function following acute oral administration of 0, 5, 10, and 30 mg eq/kg of GS-6637 using four separate groups of rats (n = 13–14/group). Next, we studied the effect of subchronic (seven consecutive days) oral gavage administration of the compound at 0, 5, 10, and 30 mg eq/kg using the same four independent groups used for the dose response study (n = 12–14/group). We completed this study with a separate group of animals by testing the efficacy of oral varenicline (Chantix) at 1.6, 3.2, and 6.4 mg/kg (n = 9/group) compared to GS-6637.

2.3. Drug preparation

Solutions of (−)-nicotine hydrogen ditartrate were prepared in pyrogen-free glassware in sterilized isotonic saline. Doses were calculated as a function of the nicotine base weight. The pH of the solutions was adjusted to 7.0 using NaOH. Next, the solutions were passed through a 0.2 µm filter (Millipore Corp, Billerica, MA, USA). Solutions of GS-6637 (Lot #8 & 9) were prepared in pyrogen-free glassware in vehicle (Lot #MG-4373-143, Formulation 2B: 25% PEG 400/5% Vit E TPGS/1% SLS, 0.5% Methocel, 69% water). GS-6637 and vehicle were provided by Gilead Sciences. Varenicline was purchased from Sigma-Aldrich (Saint Louis, MO, USA) and was dissolved in dH2O. GS-6637 and vehicle were administered orally (gavage) in a volume of 4 mL/kg one hour before testing sessions. Varenicline (4 mL/kg, PO) and its vehicle were administered 15 min before testing.

2.4. Training and surgery

Rats were placed in dual lever test chambers for behavioral training. Each chamber was equipped with a tone generator, a house light, a cue light above each lever, and a metal tether to cover the drug delivery line. A computer programmed with MED-PC software controlled experimental events and data collection. Each catheter was connected to a micro-liter syringe pump and tethers made of polyethylene tubing with huber needles for access to ports and catheters. Rats wore infusion harnesses during each session to connect them to the tethers. Initially, the rats were trained daily during 15 min with tutor sessions to press the levers for food pellet reinforcements. Half the animals were rewarded for responding on the right lever and half for responding on the left. Only the cue light over the correct lever was illuminated while the light over the incorrect lever was off. Responses on the correct lever were rewarded by pressing a button connected to the control panel, which caused immediate delivery of one 45 mg food pellet and activation of the feedback tone for 0.5 s. There were no timeout periods in the tutor sessions. These tutor sessions were followed by three daily 45 min pellet sessions on a fixed ratio 1 (FR1) schedule, where rats obtained one food pellet after pressing the lever once. The training procedure took place before rats underwent surgery for catheter implantation for IV nicotine self-administration.

Following the completion of their final training session with food reinforcement, animals were anesthetized with a mixture of ketamine (60 mg/kg) and dormitor (15 mg/kg). A plastic SoloPort was attached intraoperatively to a polyurethane catheter (Strategic Application, Libertyville, IL, USA), inserted into a subcutaneous interscapular pocket, and sutured to the underlying fascia of the rat. The following day, the rats began self-administration sessions with nicotine (0.03 mg/kg/infusion, IV) as the reinforcer. A lever press on the active side resulted in the activation of the feedback tone for 0.5 s, the immediate delivery of one 50 µL infusion of nicotine in less than 1 s. Each infusion was immediately followed by a one-minute timeout period in which the house
light turned on and cue lights turned off while responses were recorded but not reinforced. Two levers were available in the operant chamber during every session of nicotine self-administration but only pressing one of them resulted in the nicotine delivery; the other lever served as a control. The dose of nicotine was 0.03 mg/kg/infusion. An FR1 schedule was used, meaning that rats had to press the lever once to obtain one infusion of nicotine. Nicotine self-administration sessions lasted for 45 min. The catheters were flushed daily before experimental sessions with 0.3 mL of a solution of 100 U/mL heparinized saline (Baxter Health Corporation, Deerfield, IL, USA). After completion of each test session, nicotine remaining in the port was removed and a 0.3 mL sterile lock solution containing 500 U/mL of heparinized saline and 8 mg/mL of gentamicin (American Pharmaceutical Partners, Schaumberg, IL, USA) was infused [23,24,25].

2.5. EXP. 1. Acute dose-response of GS-6637 on nicotine self-administration

After acclimation, training, and acquisition of a stable nicotine self-administration, the dose-response of oral gavage administration of 0, 5, 10, and 30 mg eq/kg of GS-6637 was determined one hour before the nicotine session using four independent groups of rats (n = 13–14/group). This experiment was replicated with a one-week interval between drug administrations in the same rats. Rats received nicotine self-administration sessions five days a week.

2.6. EXP. 2. Subchronic effects of GS-6637 on nicotine self-administration

In this experiment, the same animals used for the acute dose response were used to study the effect of subchronic (seven consecutive days) oral gavage administration of GS-6637 (0, 5, 10, and 30 mg eq/kg) on nicotine self-administration. Subchronic treatment began after rats re-established baseline levels of nicotine intake. Rats were given an oral dose of 5, 10 or 30 mg/kg eq of GS-6637 or the same volume (4 mL/kg) of vehicle for seven consecutive days, one hour before nicotine self-administration session began. Following the termination of the subchronic experiment, nicotine self-administration of the rats was monitored for three extra days with no drug administration.

2.7. EXP. 3. Acute dose-response of varenicline on nicotine self-administration

A new group of rats was used to study the effect of varenicline on nicotine self-administration. As in EXP. 1, after acclimation, training, and acquisition of stable nicotine self-administration, a dose-response was studied following a single oral gavage administration of 1.6, 3.2, and 6.4 mg/kg of varenicline 15 min before nicotine sessions using a counterbalanced design (n = 9/group). This experiment was replicated with a one-week interval between drug administrations in the same rats. Rats received self-administration sessions five days a week.

2.8. Data compilation and analysis

Data were collected online simultaneously from multiple operant chambers. The number of infusions/session was recorded. Data were normally distributed and evaluated with analysis of variance (ANOVA). Acute and subchronic GS-6637 treatment data was analyzed with one-way ANOVA as four independent treatment groups (0, 5, 10, and 30 mg eq/kg) were used. Varenicline treatment data was analyzed with repeated two-way ANOVA as a counterbalanced design was used. Post-hoc Fisher test was used where appropriate. Alpha of P < .05 (two-tailed) was used as the threshold for statistical significance. Data were analyzed with Statistica software and graphed with Graphpad Prism software.

3. Results

Acute oral administration of GS-6637 at 10 mg eq/kg and 30 mg eq/kg significantly reduced nicotine self-administration when compared with vehicle (46% and 67% inhibition, resp.). One-way ANOVA showed a significant effect of treatment on the number of nicotine infusions [F(3,51) = 8.77, P < .05] (Figure 1).

Similarly, subchronic administration of GS-6637 for seven consecutive days showed a significant reduction of nicotine self-administration at 10 mg eq/kg (39% inhibition) and 30 mg eq/kg (61% inhibition) without tolerance. One-way ANOVA showed a significant effect of treatment on the number of nicotine infusions [F(3,48) = 3.32, P < .05] (Figure 2).
Table 1: Single oral administration of varenicline significantly reduced nicotine self-administration. Data represent mean ± SEM, N = 9.

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>1.6 mg/kg Chantix</th>
<th>3.2 mg/kg Chantix</th>
<th>6.4 mg/kg Chantix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of nicotine infusions</td>
<td>6.3 ± 0.9</td>
<td>4.4 ± 0.9</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>% inhibition vs. vehicle</td>
<td>31% (P = .07, N.S.)</td>
<td>52% (P = .004)</td>
<td>49% (P = .006)</td>
</tr>
</tbody>
</table>

Figure 2: Oral gavage administration of GS-6637 for seven consecutive days inhibits nicotine self-administration without inducing tolerance. Compared to the vehicle, 10 mg/kg and 30 mg/kg of the drug induced 39% and 61% inhibition in nicotine infusion, respectively. The data represent mean ± SEM, N = 12–14, P < .05.

A single oral dose of varenicline significantly reduced nicotine self-administration when compared with vehicle (repeated measure ANOVA showed a significant effect of treatment on number of nicotine infusions \( F(3, 24) = 4.20, P < .05 \); 3.2 mg/kg (52% inhibition) and 6.4 mg/kg (49% inhibition); \( P = .004 \) and \( P = .006 \), resp.; Fisher post-hoc test). However, the lowest dose of 1.6 mg/kg was not as effective (31% inhibition, \( P = .07 \), N.S.; Fisher post-hoc test) (Table 1).

4. Discussion and conclusions

Currently available medications to stop smoking and prevent relapse are only modestly effective [19]. The major finding in this study is that a highly selective inhibitor of ALDH-2 (GS-6637) given orally reduces nicotine self-administration without inducing tolerance to the medication. Thus, selective ALDH-2 inhibitors alone or in combination with other agents could provide a novel therapeutic avenue for more effective treatment of nicotine intake. The current study shows that ALDH-2 inhibition significantly reduces nicotine self-administration in rats. This is consistent with previous studies showing that selective inhibition of ALDH-2 with a different compound, CVT-10216 (3-((3-(4-(methylsulfonamido)phenyl)-4-oxo-4H-chromen-7-yloxy)methyl)benzoic acid), suppresses cocaine self-administration in rats [20] and anxiety [26] as well as alcohol intake in alcohol preferring Fawn-Hooded rats [21]. Taken together, these findings suggest that, in general, a class of selective ALDH-2 inhibitors would be expected to suppress addictive drug-seeking behaviors.

The mesocorticolimbic dopaminergic system is implicated in the reinforcing properties of abused substances including nicotine [5,6,7,8,15]. The rewarding effects of cocaine and alcohol have also been attributed to DA release in the nucleus accumbens [7,15]. In this study, the efficacy of ALDH-2 inhibition in reducing nicotine self-administration is likely due to a reduction in DA production as reported with cocaine [20]. Additionally, the demonstration that nicotine also can block DA reuptake [17] supports the hypothesis that the dopaminergic systems in the brain play a major role in nicotine reinforcement. Thus, we propose that inhibition of nicotine-induced DA release in the nucleus accumbens by selective ALDH-2 inhibitors appears to reduce the reinforcing effects of nicotine in addicts.

The relationship of ALDH-2 inhibition to nicotine self-administration can be summarized as follows: dopamine is synthesized in the VTA neurons and released in the nucleus accumbens [27,28,29]. It has been shown that ALDH-2 plays a role in DA metabolism in the brain [30]. The mechanism by which ALDH-2 inhibition reduces DA production involves several steps in DA metabolism. Tyrosine hydroxylase (TH) is activated during drug taking resulting in increased DA synthesis. MAO enzyme converts DA to 3,4-dihydroxyphenylacetaldehyde (DOPAL), a neural substrate for ALDH-2. Therefore, as a consequence of ALDH-2 inhibition, DOPAL is not metabolized by ALDH-2 but instead becomes available to condense with DA to form tetrahydrodopamine (THP) [31]. THP is a highly selective inhibitor of activated TH, the rate-limiting step in DA synthesis in the VTA [20,32]. Thus, acting as an endogenous negative-feedback inhibitor generated in the presence of GS-6637, THP attenuates drug-induced DA synthesis [20].

However, it should be noted that even though dopaminergic systems in the brain play a major role in the reward properties of some drugs of abuse, DA is not the only neurotransmitter to be involved in drugs reinforcement. There is enough neurochemical and pharmacological evidence to support the involvement of other neurotransmitters such as cholinergic, glutamatergic, GABAergic, histaminergic, serotonergic, and endogenous opioid systems in drug-taking.
behaviors [27, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42]. Nevertheless, there is evidence that the final common mechanism of action of most drugs of abuse often involves DA.

For clinical utility, it is important to show that GS-6637 does not induce tolerance with continuing use. We find that GS-6637 given daily for seven days reduced nicotine self-administration in rats without lessening efficacy. It is also clinically important that GS-6637 is effective when given orally. Moreover, the efficacy of GS-6637 in reducing nicotine intake appears to be comparable with varenicline in rats. In conclusion, our findings demonstrate that both acute and subchronic treatments with a selective ALDH-2 inhibitor significantly reduce nicotine self-administration without inducing tolerance. This suggests that selective reversible ALDH-2 inhibition appears to have therapeutic potential for treating nicotine addiction.

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Conflict of interest The authors declare that they have no conflict of interest.

References


