A novel selective ALDH-2 inhibitor exhibits anti-drinking, anti-addictive and anxiolytic properties
Evidence for a new pharmacology of action

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- Maria Pia Arolfo

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**Contractor**
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  - San Diego

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- David Overstreet, University of N. Carolina (Chapel Hill)
- Peter Kalivas and Ryan LaLumiere, Medical Univ. of S. Carolina at Charleston
- Andrew Lawrence, Florey Inst., Melbourne
- Emanuel Rubin and Helen Anni, Jefferson. Phila
Alcoholism in the US is Common and Costly

- 7-10% of Americans are Alcoholics
- Cost to society: $185 billion (est)
- 15% - 25% Healthcare Budget
- 20 – 36 % primary care patients
Alcoholism

Heavy drinking causes harm

*Harm reduction* is good medicine

Current Treatment Goals: convert excessive drinking to moderate drinking and prevent harm
Most alcoholics are not treated with medications

We can do **better**
An important clue comes from human genetics
ALDH-2 and Alcoholism

• South-East Asians with ALDH-2 deficiency (*487Lys mutation) are protected from alcoholism
• Heterozygotes (approx 40%) with reduced ALDH-2 activity and a “flushing reaction” when drinking have a reduced incidence of alcoholism
• Individuals with homozygous expression (approx 5%) do not become alcoholics
• ALDH-2 knockout or silencing inhibits excessive drinking in rodent models of alcoholism
Another important clue comes from ancient Chinese herbal medicine
History of Treatments for Alcohol Abuse/Dependence

WEST

EAST

Kudzu
-Li Dongyuan

Kudzu flower/Root

Disulfiram

Acamprosate

Naltrexone

WM Keung
Daidzin is an Anti-Drinking Principle of Kudzu (*Pueraria lobata*)

**Root**
**Radix puerariae, RP**

![Daidzin](image)

*WM Keung*
Daidzin is a reversible competitive inhibitor of ALDH-2 and not ALDH-1

MW Keung
Kudzu Extract or Daidzin Inhibits Alcohol Consumption

<table>
<thead>
<tr>
<th></th>
<th>Kudzu Extract</th>
<th>Daidzin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.p.</td>
<td>p.o.</td>
</tr>
<tr>
<td>Golden hamsters\textsuperscript{a}</td>
<td>▼</td>
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<tr>
<td>Rats</td>
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<tr>
<td>Outbred Wistar\textsuperscript{a}</td>
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<tr>
<td>Fawn Hooded\textsuperscript{b}</td>
<td>▼</td>
<td>▼</td>
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<tr>
<td>P\textsuperscript{a,b,c}</td>
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<td></td>
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<tr>
<td>sP\textsuperscript{d}</td>
<td></td>
<td></td>
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<tr>
<td>HAD\textsuperscript{b}</td>
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<tr>
<td>Monkey\textsuperscript{b}</td>
<td></td>
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<tr>
<td>Human Heavy drinkers\textsuperscript{e}</td>
<td></td>
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<tr>
<td>Chronic alcoholics\textsuperscript{f}</td>
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</tbody>
</table>

\textsuperscript{a}Keung et al. 1993, 1996; \textsuperscript{b}Overstreet et al. 1996; \textsuperscript{c}Lin et al. 1996; \textsuperscript{d}Perfumi et al. 1998; \textsuperscript{e}Lukas et al. 2005; \textsuperscript{f}Shebek et al. 2000.
Enter CV Therapeutics
(now Gilead Sciences)
ALDH2 Chemistry Program at CVT Structure Based Design
Ligand - Receptor Interactions
H Bonds, p-p Stacking, Possible Salt Bridge

Polar Pocket
phenol

Daidzin X-Ray
Sugar

\( \pi - \pi \) Stacking
Lys 127
Small Molecule ALDH-2 Drug Discovery at CVT

Structure Based Design
Harvard (WM Keung) Collaboration

Synthesis 100’s Compounds

Selective ALDH-2 Inhibition

Good Pharmaceutical Properties

25 Compounds Efficacy

CVT-10216
Prevailing View Based on ALDH-2 Deficiency

Increased acetaldehyde discourages continued drinking because of adverse effects

How to study in rodents?
Humans

sucrose + ethanol → ethanol

Rodents

Tricia Janak
A single dose of CVT-10216 (i.p.) inhibits ethanol drinking in FH rats (2 bottle choice)

% Inhibition as a Function of Time and Dose

D. Overstreet

n = 9

- Baseline
- 7.5 mg/kg
- 15 mg/kg
- 30 mg/kg

* p<0.05
** p<0.01
CVT-10216 Does not Reduce Water Consumption in FH Rats

D. Overstreet
Deprivation-Induced Drinking

• Heavy drinking FH rats are denied alcohol for 5 days
• Alcohol then made available
• Rats now exhibit deprivation-induced increased drinking, considered evidence for stress-induced craving

We asked whether a single dose of CVT-10216 would prevent deprivation-induced drinking
CVT-10216 Prevents Deprivation-Induced Drinking in FH Rats After 5 Days of Abstinence

CVT-10216 (15 mg/kg)

n=6
Untreated
Vehicle
CVT-10216

* p< 0.05
** p< 0.01

D. Overstreet
Ethanol-Reinforced Operant Self-Administration

10% Ethanol

10% Ethanol reward after 3 presses

Inactive
CVT-10216 30 min. before testing prevents alcohol self-administration in LE Rats

LE rats at the “Bar”
30 min. to work for a drink every 24 hrs

EtOH intake (g/kg)

MP Arolfo, C. Ou

GILEAD
CVT-10216 also decreases EtOH self-administration in iP rats

Notice “shift to left” for dose-response

A. Lawrence
CVT-10216 prevents cue-induced lever pressing even when alcohol is not delivered

No alcohol for 24 hrs = No Acetaldehyde
Conclusions

• CVT-10216 reduces
  – heavy drinking and relapse
  – deprivation/stress-induced drinking
  – self-administration and reinstatement

A single dose of CVT-10216 appears to safely reduce heavy drinking and EtOH seeking in rats

Acetaldehyde is not necessarily required
CVT-10216 targets alcohol-related behavior

How does this happen if acetaldehyde not necessarily required?

Clue: Alcohol Activates the Nucleus Accumbens
Self-Stimulation of the Nucleus Accumbens Replaces Normal Desires
Alcohol and All Addicting Drugs Activate the Nucleus Accumbens
CVT-10216 and the Nucleus Accumbens

- The nucleus accumbens (NAc) contributes to craving and the reward of addicting drugs
- Increased dopamine (DA) in the nucleus accumbens is implicated in NAc responses to addicting drugs

We asked whether CVT-10216 affects DA levels in the NAc
CVT-10216 prevents ethanol-induced increases in NAc dopamine

**Diagram Description:**

- **Y-axis:** Dialysate [DA] (% of Baseline)
- **X-axis:** Time (hr)
- **Graph Lines:**
  - Vehicle (gray)
  - 7.5 mg/kg (yellow)
  - 15 mg/kg (orange)

- **Key Points:**
  - CVT injection at 0.5 hr
  - ETOH (ethanol) injection at 1.5 hr

**Additional Text:**

F. Olive

GILEAD
CVT-10216 does not reduce basal levels of dopamine in rat Nucleus Accumbens (i.e. normal functions OK)

Dialysate [DA] (% of Baseline) Time (hr)

0.0 0.5 1.0 1.5 2.0 2.5

CVT 050 100 150 200 250

Vehicle (n=4)
7.5 mg/kg (n=6)
15 mg/kg (n=6)

F. Olive
Craving and alcohol use appears to be associated with increased DA levels in the NAc and sensitivity to inhibition by CVT-10216.

Basal dopamine levels are unaffected.

**Prediction:**

Moderate drinking without physical dependence may be less sensitive to CVT-10216.
Moderate drinking is not affected by CVT-10216 in LE Rats (2 bottle choice)

M.P Arolfo, C. Ou
Increased Dopamine in the Nucleus Accumbens Appears to Characterize all Addictions

What about CVT-10216 and other addicting drugs?
Relapse is the most serious limitation of effective medical treatment of alcoholism and all addictions.
Relapse to I.V. Drug Use Following Termination of Treatment

Ball & Ross, 1991 (Adapted)
Alcoholics commonly use cocaine
Cocaine increases dopamine

We asked whether CVT-10216 would also prevent cocaine reinstatement (relapse)
Assay for i.v. Cocaine Relapse

- # Lever Presses for I.V. Cocaine
- Cocaine Available
- Cocaine not Available
- Cocaine (10 mg/kg i.p.)

H. de Wit

Acquisition/Maintenance | Extinction | RELAPSE
A Single Dose of CVT-10216 (i.p.) Prevents Cocaine Relapse

MP Arolfo
CVT-10216 also Prevents Cue-induced Cocaine Relapse Weeks after Last Dose

MP Arolfo, C. Ou
CVT-10216 Prevents Heroin Relapse

- CVT-10216 Prevents Heroin Relapse
- Extinction: 0.06 mg x 3 hrs
- Priming: 0.25 mg/kg s.c.

**R. Lalumiere, P. Kalivas**

Number of lever presses

<table>
<thead>
<tr>
<th>CVT-10216 (mg/kg)</th>
<th>Extinction</th>
<th>Vehicle</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=14</td>
<td>n=14</td>
<td>n=7</td>
<td>n=7</td>
</tr>
</tbody>
</table>

- # Significantly different from Extinction, p<0.05
- *Significantly different from Vehicle, p<0.05

Heroin: 0.06 mg x 3 hrs

GILEAD
CVT-10216 also prevents Nicotine Self-Administration

Study #1

Study #2

M. Azar
A highly selective reversible inhibitor of ALDH-2 suppresses heavy drinking and addictions

Does ALDH-2 inhibition affect behaviors related to drug-seeking and craving?
Stress and Relapse

Stress (anxiety) often precipitates relapse in alcoholics and drug addicts

We next asked whether CVT-10216 has anxiolytic properties
We asked whether CVT-101216 would prolong social interaction (reduce anxiety)
Anxiety Models

- Genetic – e.g. innate anxiety in FH rats
- Repeated Alcohol Withdrawal
- Stress-Induced anxiety
- Drug-induced anxiety
CVT-10216 (15 mg/kg) Increases Social Interaction in Naive FH Rats

* p< 0.01

D. Overstreet
Repeated EtOH Withdrawal in SD rats

- 4.5% EtOH diet for 5 days, followed by 2 days of withdrawal X3 cycles (3 weeks)
- Social interaction and motor activity assayed 5 hr after drinking
- CVT-10216 administered once after the 3rd cycle 30 min. before testing (Acute) or 2x in the 1st and 2nd cycles followed by an untreated 3rd cycle (Prophylactic)

D. Overstreet
Acute CVT-10216 (15 mg/kg) Counteracts Alcohol Withdrawal-Induced Anxiety in SD Rats

N=8  *p < 0.01

D. Overstreet
Prophylactic CVT-10216 Counteracts the Development of Withdrawal-Induced Anxiety in SD Rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Social Interaction (Sec)</th>
</tr>
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<tbody>
<tr>
<td>Control Diet</td>
<td>22</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9</td>
</tr>
<tr>
<td>CVT 3.75</td>
<td>17**</td>
</tr>
<tr>
<td>CVT 7.5</td>
<td>20**</td>
</tr>
<tr>
<td>CVT 15</td>
<td>20**</td>
</tr>
</tbody>
</table>

* Significantly different from Control Diet p < 0.01
** Significantly different from vehicle, p < 0.01

D. Overstreet
Restraint-induced Stress

• Rats are restrained for one hour
• Then returned to home cages for 15 min
• Next assayed in the open field arena for 5 min
• CVT-10216 given i.p. 30 min. before testing

D. Overstreet
Acute CVT-10216 (15 mg/kg) Counteracts Restraint-induced Anxiety in SD Rats

Social Interactions (sec)

Control/Vehicle (N = 8)  
Vehicle (N = 8)  
CVT-10216 (N = 8)  

*Np < 0.01

D. Overstreet
CVT-10216 Prevents Anxiety Produced by a Benzodiazepine Inverse Agonist (But does not correct 5HT2C antagonist-induced anxiety)

*Significantly different, p < 0.01, from VEH-VEH
+Significantly different, p < 0.01, from VEH-DMCM

Overstreet, D.
CVT-10216 inhibits desire for heavy drinking without appearing to affect moderate drinking.

The greater the urge, the more effective CVT-10216 (e.g., relapse).

CVT-10216 also appears to have anxiolytic properties.

CVT-10216 reduces addictive drug seeking and relapse.

CVT-10216 represents a new generation of possible medications for treating alcoholism, other addictions, and anxiety.
• Back up slides
Acamprosate: Newest Approved Drug, Minor Benefit

NMDA Receptor Antagonist (1-2 gm/day)

- Approved and marketed in U.S.
- Pooled data of double-blind studies (>3000)
- Twice as many patients who received acamprosate vs. placebo remained abstinent (like naltrexone).
- Many studies, not all positive
- Helpful but minor effect
- No benefit adding acamprosate to naltrexone