Pharmacogenetic Approaches to the Treatment of Alcoholism: Preclinical Studies

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Factors that influence the Probability of Developing Alcoholism

- Genetics: 60%
- Psychosocial: 40%

- Price
- Availability
- Permissiveness
- Recognition of Risk

- Identical vs Fraternal twins
- Children adopted at an early age.
- SNPs in the genome of affected families
- Genetic protection in Asians: AVERSION
ONCE ALCOHOLISM HAS DEVELOPED it is maintained by:

Positive Reinforcement
(rewarding effects and no aversive effects)

Negative reinforcement
(reducing withdrawal symptomatology)

Conditioning (situational)
(memory, stress: “Craving”)
TREATMENT DEALS MOSTLY WITH:

- Positive Reinforcement
  (reduce rewarding effects; increase aversive effects)

- Negative reinforcement
  (reducing withdrawal symptomatology)

- Conditioning (situational)
  (memory, stress: "Craving")
ANIMAL MODELS FOR ALCOHOLISM THERAPIES

Positive Reinforcement
(increase aversive effects: reduce rewarding effects)

Conditioning (situational)
(Memory, stress, psychotherapy)
Metabolism of Alcohol

Deshidrogenasa alcohólica (ADH) catalyzes the conversion of ethanol (CH₃CH₂OH) to acetaldehyde (CH₃CHO), which is then converted to acetate (CH₃COO⁻) by Deshidrogenasa aldehídica (ALDH2) using NAD⁺ and NADH.

Disulfiram inhibits ALDH2, leading to accumulation of acetaldehyde and an aversive reaction.
NATURE’s DISULFIRAM: Inactivating mutation of ALDH2: an effect analogous to that of disulfiram
Reduction of Alcoholism prevalence in Asia:
ALDH2 +/- = - 67%
ALDH2 --/- = - 99%

Alcohol Dehydrogenase (ADH)

Aldehyde Dehydrogenase (ALDH2)

NAD^+ to NADH to NAD^+ cycle

30% of Asians
Selective breeding of RATS:

UChA (abstainers) y UChB (bibulous)

Alcohol (10%) and water available 24 hours/day

Drinker rats
Chile, Finland, USA, Italy
(Equivalent 1 liter whiskey day)

Aldh2*1/Aldh2*1

Aldh2*2/Aldh2*2
ABSTAINER RATS ALSO SHOW ELEVATIONS IN BLOOD ACETALDEHYDE LEVELS WHEN ADMINISTERED ETHANOL

Etanol (1g/kg)
Antisense molecules bind to the mRNA like a magnet, blocking the gene message. Gene coded anti ALDH2. ALDH2 is not produced.
Generation of an Adenoviral Vector携带一个anti-ALDH2 antisense基因
(Troyan Horse)

![Image of Adenoviral Vector and DNA](image_url)

- **ITR**
- **Ψ**
- **anti-ALDH2 antisense**
- **Adenoviral genes E2-L4**
- **ITR**

Liver specific 70nm
Preferential entry of Adenoviral vectors into liver cells (hepatocytes)

Fenestra: (pores) 300 – 1000 nm. Adenovirus: 70 nm; Other capillaries: <20nm

Micrograph of Robin Fraser, University of Otago, New Zealand)
Effect of i.v. antisense gene anti ALDH2 on ALDH2 activity and blood acetaldehyde

Arterial acetaldehyde following Ethanol 1g/kg i.p.

Liver ALDH2 activity

<table>
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<th>Minutes</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
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<tr>
<td>Arterial acetaldehyde (µM)</td>
<td>0</td>
<td>40</td>
<td>80</td>
<td>60</td>
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- p<0.02
- p<0.01
- NS

AdV-AS

Viral vehicle

ADH2 activity (nmol/min/mg)

AdV-control

AdV-AS

p<0.002

**
UChB rats allowed alcohol consumption for two months.

Antisense anti ALDH2 generated by adenovirus (AdV-cDNA ALDH2-AS)

Free access to alcohol (10%) and water

2 months

Happy hour 10% alcohol and water

Without alcohol

35 days

3 días
Proof of Principle:
Happy-hour by alcohol-dependent UChB rats
(after 2 months of ethanol free choice)

One Hour Alcohol Consumption (g/kg/h)

Days

AdV-control

AdV-anti ALDH2

-50%; p<0.001
Acetaldehyde: A NEW FRONTIER

**Aversion:**

- Acetaldehyde produced in the liver

**Reward:**

- Acetaldehyde in the CNS
Does Acetaldehyde cross the blood-brain barrier?

**NO:**

At the levels of acetaldehyde present in blood, the enzyme ALDH2 in the tight-junction endothelial cells of brain capillaries oxidizes acetaldehyde into acetate.
Acetaldehyde: A NEW FRONTIER

Reward:

Acetaldehyde in the CNS

Aversion:

Acetaldehyde produced in the liver
The dopaminergic neurons in the ventral tegmental area project neuronal axons into the nucleus accumbens releasing dopamine.
The other side of the Coin

Recent literature indicates that in the brain acetaldehyde is not aversive but rather reinforcing.

Rat bred as alcohol drinkers (Indianapolis P rats) will self-administer acetaldehyde into the dopaminergic neurones of the ventral tegmental area of the brain (VTA):

(Concentration needed to promote self administration)

- Ethanol 0.02 M
- Acetaldehyde 0.00005 M

Rodd et al, Alcoholism Clinical and Experimental Research 2005; 2008
Is ethanol metabolized into acetaldehyde in the brain?

**YES**

Although there is no alcohol dehydrogenase in the brain, catalasa and CYP2E1 present in the brain are able to metabolize ethanol.

There is also an active brain aldehyde dehydrogenase.

Then let's
(a) lower the activity of catalase or
(b) increase activity of aldehyde dehydrogenase
Inhibition of catalase synthesis by microinjection of Lentiviral vector coding for shRNA anticatalase into the VTA

Injection of Lentiviral Vector (gutless)

shRNA anticatalase Lenti

Dopaminergic neurones (VTA)

Inhibition of catalase synthesis by microinjection of Lentiviral vector coding for shRNA anticatalase into the VTA
Inhibition of Brain Catalase (VTA) by stereotaxic administration of an anti-catalase viral vector.
Inhibition of alcohol consumption by inhibition of brain acetaldehyde synthesis (lowering catalase synthesis)
Anticatalase Lentiviral vector specifically blocks the liberation of dopamine in Nucleus Accumbens

Karahanian E et al ePub 2011
Inhibition of alcohol consumption by activation of brain acetaldehyde elimination (by increasing aldehyde dehydrogenase synthesis)

Control vector

Aldehyde dehydrogenase vector (ALDH2)

Manuscript in preparation 2012
TREATMENT DEALS MOSTLY WITH:

Positive Reinforcement
(increasing aversive effects or reduce rewarding effects; )

Conditioning (situational)
(memory, stress: “Craving”)
Partial extinction of conditioning is needed to observe the inhibitory effect of the anticatalase lentiviral vector on ethanol intake.
Effect of Anticatalase on the Alcohol deprivation effect (ADE) “happy hour”

80% inhibition of “binge drinking” by brain (VTA) anticatalase
GENE DELIVERY
CONCLUSIONES TO TREAT ALCOHOLISM
FROM PRECLINICAL STUDIES

1. Inhibit acetaldehyde degradation.
   By reduction of liver aldehyde dehydrogenase gene expression
   (duración 30 days-1 year)

2. Reduce brain catalase synthesis by gene delivery.
   Inhibition of brain acetaldehyde generation.

3. Increase brain aldehyde dehydrogenase synthesis by gene delivery.
   Activation of brain acetaldehyde degradation

4. Reduce conditioning and memory of reward (added to the above).
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