2021

In vitro comparison of Ethanol Metabolism in Precision Cut Liver Slices from C57Bl/6, Balb/c, DBA/2J and 129S1/SvlmJ Mice and with the Aldeyra Product ADX-629

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Summer Undergraduate Research Program

In vitro comparison of Ethanol Metabolism in Precision Cut Liver Slices from C57Bl/6, Balb/c, DBA/2J and 129S1/SvlmJ Mice and with the Aldeyra product ADX-629

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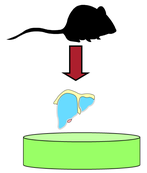
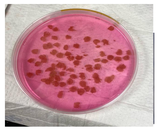
Introduction & Background

- Excessive consumption of alcohol can lead to alcoholic fatty liver disease. Development of this disease is due to the byproducts of ethanol metabolism. These byproducts include acetaldehyde (from ethanol) and malondialdehyde (from the breakdown of cell membranes during injury) The Aldeyra product, ADX-629, is a small molecule that is a reactive aldehyde species (RASP) inhibitor that covalently binds free aldehydes and diminishes excessive RASP levels. However, the ability of ADX-629 to inhibit these aldehyde metabolites generated from ethanol metabolism has yet to be determined.
- Precision Cut Liver Slices (PCLSs) provide a novel *in vitro/ex vivo* model for studying the effect of alcohol exposure as these cells will be under a controlled environment and exposed to equal levels of alcohol over time.
- Previous studies have shown that PCLSs:
  - From rodents metabolize ethanol and remain viable over 4-5 days
  - Incubation with 25 mM ethanol (EtOH) results in fibrosis that is associated with ethanol metabolism that:
    - Produces pro-fibrotic molecules
    - Releases pro-inflammatory cytokines

Methods





1. Female Mice (C57/BL6) were anesthetized using isoflurane
2. Livers were removed and placed on a V7 preservation buffer.
3. Cylindrical tissue cores (8 mm) were cut using a hand-held coring tool
4. Cores were then loaded into the Vitron tissue slicer and cut to a 250-µm thickness
5. Slices were placed in Williams E medium containing D-glucose and gentamicin (WEGG) under 95% O2-5% CO2 (carbogen) at 37 degrees Celsius for 30 min.
6. Slices were floated onto a titanium screen containing rollers from Vitron. These rollers were inserted into sterile 20-ml glass vials containing 1.7 ml of serum-free WEGG medium or WEGG medium containing 25 mM ethanol.
7. Treatment of PCLS with ADX-629 in a dose dependent manner.

Aim & Purpose

The purpose of this study was to determine if aldehyde scavenger, ADX-629, could prevent the deleterious effects of ethanol caused by its metabolites. Also, to evaluate the effects of this drug on triglyceride formation.

Hypothesis

The use of ADX-629 will attenuate the effects on the liver from aldehydes and will reduce the formation of a fatty liver

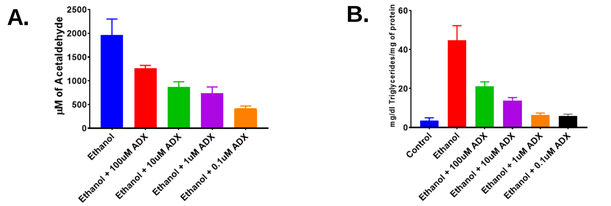


Figure 1. ADX-629 Dose Response. PCLS were treated with ethanol (25 mM) and incubated with various concentrations of ADX for 24 hours. **(A)** Acetaldehyde (AA) levels following treatment with lowering doses of ADX. A significant drop in AA was demonstrated with the lower doses of ADX. (**B**) Triglyceride levels following treatment with lowering doses of ADX. A significant drop in triglyceride levels is demonstrated with the lower doses of ADX. Data strongly suggests that higher doses of the ADX cause an incomplete response as compared to the lower doses. Even lower levels of ADX need to be evaluated. N=6

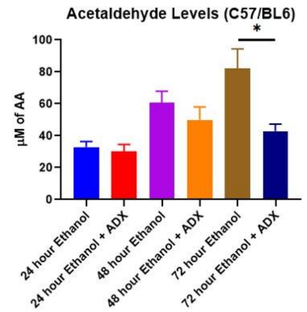


Figure 2. Acetaldehyde levels following Treatment of PCLS with 1µM of ADX-629. PCLS were incubated with ethanol in the presence of 1µM of ADX for 24,48, and 72 hours. ADX reduced the levels of AA at 24 and 48. However, significance was not reach in this experiment. The 72-hour time point did significantly reduce AA levels *p<0.01. N=9

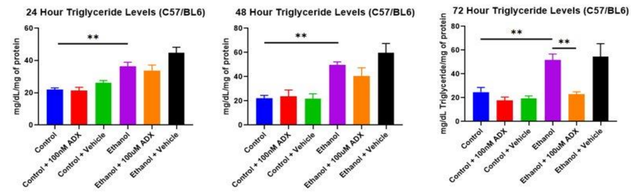


Figure 3. Triglyceride levels following Treatment of PCLS with 1µM of ADX-629. PCLS were incubated with ethanol in the presence of 1µM of ADX for 24,48, and 72 hours. **(A)** Triglyceride levels were significantly increased p<0.001 compared to control treated PCLS at 24 hours. **(B)** Triglyceride levels were significantly increased p<0.001 compared to control treated PCLS at 48 hours. (C) Triglyceride levels were significantly increased p<0.001 compared to control treated PCLS at 72 hours. However, ADX treatment significantly reduced p<0.001 these levels back to control values. N=9

Results

- Treatment of PCLS with Ethanol and ADX significantly reduced:
  - The levels of acetaldehyde released into the media in a dose fashion
- The use of 1µM ADX reduced the:
  - Acetaldehyde levels in the supernatant over time. However, 72 hours was the only significant value present.
  - Triglyceride production over time. However, 72 hours was the only significant value present.
- There appears to be an interaction with high levels of ADX-629 and PCLSs in which the drug is not efficacious. Interestingly the lower levels are more effective.
- The fact that 1 and 0.1 µM of ADX-629 effectively reversed the effects of ethanol on the PCLSs demonstrates the end point concentration at which this drug works has not been reached. That is, further dilution should show an increase in acetaldehyde and triglyceride levels at which point the most effective dose can be established.
- Lactate dehydrogenase and adenosine triphosphate levels demonstrated the cell survival to be better in the presence of ADX compared to control and ethanol treated PCLS (Data not shown).

Conclusion

- ADX may be a method to reduce fatty liver formation in patients with Non-alcoholic steatohepatitis
- Treatment of alcoholic patients with ADX may prevent the formation of oxidative stress caused by the metabolic breakdown of ethanol. Thereby, preventing alcoholic liver disease.

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