Summer 8-12-2021

Alcohol in dementia: Implications of Alzheimer's like pathology in alcohol abuse

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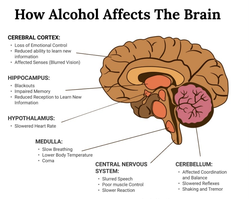
Abstract

Alcohol use is widespread, with 85.6 percent of people ages 18 and older reported drinking alcohol in their lifetime in 2019. Mild to heavy alcohol use is associated with multi-organ dysfunction at the cellular and genetic levels, as well as epigenetic modifications, leading to liver and brain damage and dementia. The role of astrocytes as contributors to Alzheimer's like pathology associated with cognitive decline in opiate abusers and people with HIV-associated neurological disorders (HAND) has been recently reported from our group. We hypothesize that alcohol could also induce astrocytic amyloidosis. In this study we demonstrated that exposure of human primary astrocytes (HPA) to ethanol resulted in a dose dependent (6.25-200 mM) increase in AD markers-amyloid precursor protein (APP), Aβ 1-42, β-site cleaving enzyme (BACE1), as well as the inflammatory marker IL1β and lncRNA BACE1AS. Next, we demonstrated a time-dependent (0-96h, 12.5 mM) upregulation of AD markers, oxidative stress (4-HNE), alcohol metabolizing enzymes alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH2), and cytochrome P450 2E1 (CYP2E1), as well as proinflammatory cytokines (TNF-α, IL1β, IL6) in HPAs exposed to alcohol. Gene silencing approaches confirmed the regulatory role of lncRNA BACE1-AS in amyloidosis and its interaction with alcohol metabolic pathways leading to neuroinflammation and oxidative stress. Further, *in vivo* study validated our *in vitro* findings, demonstrating up-regulation of APP, Aβ1-42, 4-HNE and IL1β in the cortices of ethanol-fed mice (4- weeks, ad libidum) compared to saline controls. This is the first report implicating the role of lncRNA BACE-AS in alcohol-mediated induction of astrocytic amyloidosis, leading to neuroinflammation and oxidative stress, which, in turn, could contribute to cognitive impairments. These findings set the stage for future development of therapeutic strategies aimed at targeting cognitive deficits in alcohol users and abusers.

Introduction

- According to the 2019 National Survey on Drug Use and Health, 85.6 percent of people aged 18 and older reported drinking alcohol in their lifetime and 25.8 percent reported binge drinking in the past month (SAMHSA, 2019).
- In a 25-year longitudinal study, binge drinking was associated with an odds ratio of 3.9 for dementia (Jarvenpaa et. al., 2005), additionally, light to moderate alcohol consumption was also linked with lower levels of dementia (Sabia, 2018) and amyloid buildup in the cerebrum of the brain (Kim et al., 2019).
- Previous studies demonstrate potential links between alcohol and Alzheimer's Disease (AD) e.g a recent study have shown that alcohol exposure to microglia resulted in reduction of it's phagocytic activity to remove amyloids, resulting in microglial activation and inflammation (Kalinin, 2018).
- Long noncoding RNAs (lncRNA) are involved in epigenetic, transcriptional, post-transcriptional, translational and posttranslational modification contributing to various neurological disorders including alcohol use disorder and dependence (McMichael et al., 2019) as well as AD (Luo et al., 2016).
- BACE1 antisense (BACE1AS) long noncoding RNA has been reported to facilitate amyloid production via regulation of BACE1 in AD (Zeng et al., Li et al., 2019), as well as recent reports from our lab has shown its role in AD pathology in HIV (Sil et al., 2020).
- Legal limits of alcohol in US is 0.08 gm% = 17 mM. 4 glasses of wine/ beer equivalent to ~ 10 mM alcohol concentration in blood (Koob et al., 2014).



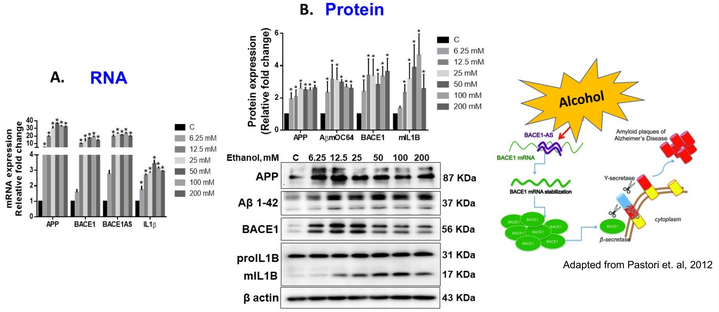
In the present study we assessed the role of alcohol in AD like phenotype leading to cognitive impairments, and the potential role of BACE1AS in this process.

Hypothesis

Ethanol exposure to astrocytes results in upregulated expression of lncRNA BACE1-AS, increased amyloidosis and dysregulated alcohol metabolism, ultimately culminating in increased oxidative stress, neuroinflammation and accompanying cognitive impairment.

Results

Dose dependent effects of Ethanol on Alzheimer's like pathology in astrocytes



Adapted from Pastori et. al, 2012

Figure 1. Ethanol induced dose response changes in human primary astrocytes (HPA): Alcohol induces mRNA expression of APP, BACE1, BACE1AS and IL1B by qRT-PCR (A). Representative western blot images for the protein expression of APP, Aβ 1-42, BACE1, and IL1B (B). Data are expressed as mean ± SEM. n= 3. Statistics: One Way ANOVA followed by Bonferroni post hoc test in Graphpad Prism software (Version 6).

Time dependent effects of Ethanol on Alzheimer's like pathology in astrocytes

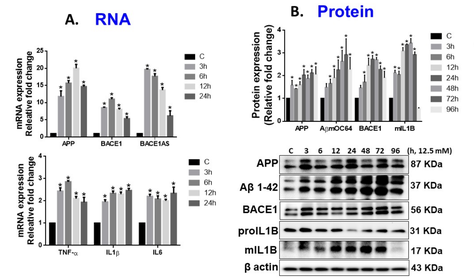
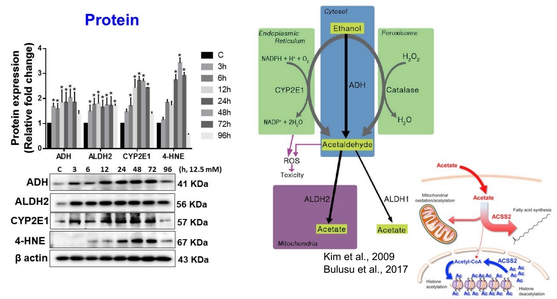


Figure 2. Ethanol induced time dependent changes in in HPA. Alcohol induces mRNA expression of APP, BACE1, BACE1AS, TNF-α, IL1B, and IL-6 by qRT-PCR (A). Representative western blot images for the protein expression of APP, Aβ 1-42, BACE1, and IL1B (B). Representative immunocytochemistry images demonstrating increased Aβ 1-42 expression ethanol exposed HPA compared to control (C), scale bar: 10 μm. Data are expressed as mean ± SEM. Statistics: n= 3. One Way ANOVA followed by Bonferroni post hoc test in Graphpad Prism software (Version 6).

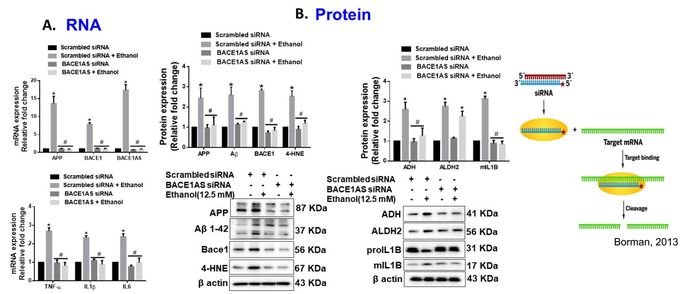
Time dependent effects of Ethanol on alcohol metabolism in astrocytes



Kim et al., 2009
Bulusu et al., 2017

Figure 3. Ethanol induced time dependent changes in alcohol metabolism in HPA. Representative western blot images for the protein expression of Alcohol Dehydrogenase (ADH), Aldehyde Dehydrogenase 2 (ALDH2), cytochrome P450 2E1 (CYP2E1), and 4-HNE (4- hydroxynonenal). Data are expressed as mean ± SEM. Statistics: One Way ANOVA followed by Bonferroni post hoc test in Graphpad Prism software (Version 6).

Role of BACE1-AS on ethanol induced Alzheimer's like pathology in astrocytes



Borman, 2013

Figure 4. Silencing of lncRNABACE1-AS inhibits alcohol-induced upregulation of Alzheimer's like pathology and dysregulated alcohol metabolism. Silencing of BACE1-AS long noncoding RNA inhibits alcohol-induced mRNA upregulation of APP, BACE1, BACE1AS, TNF-α, IL1B, and IL-6 by qRT-PCR (A). Representative western blot images for the protein expression of APP, Aβ 1-42, BACE1, 4-HNE, ADH, ALDH2, and IL1B in scrambled and BACE1AS silenced cells. Data are expressed as mean ± SEM. n=3. Statistics: One Way ANOVA followed by Bonferroni post hoc test in Graphpad Prism software (Version 6).

Alcohol induced Alzheimer's like pathology in ethanol fed mice

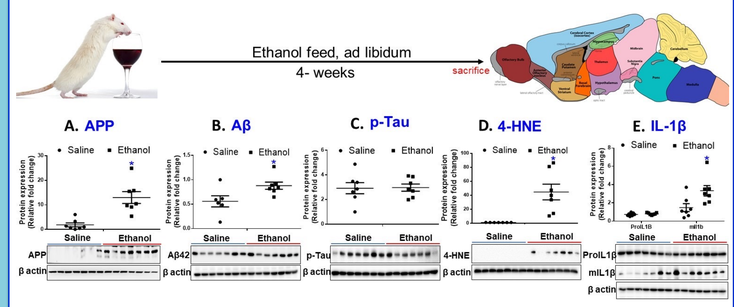


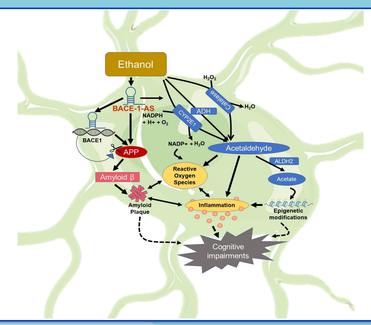
Figure 5. *Invivo* validation of Ethanol induced Alzheimer's like pathology in mice. Ethanol induced protein expression of APP (A), Aβ (B), 4-HNE (D), and mIL-1β (E), in the cortex compared to saline. No significant changes in the expression of p-Tau was observed between saline and ethanol groups (C). Data are expressed as mean ± SEM. n= 7/ group. Statistics: Unpaired student's t- test in Graphpad Prism software (Version 6).

Summary

- Ethanol exposed HPAs dose-dependently increased AD markers- amyloid precursor protein (APP), Aβ 1-42, and BACE1, as well as inflammatory cytokine IL1β and lncRNA BACE1AS.
- Ethanol exposed HPAs time-dependently increased AD markers, oxidative stress- 4-HNE, alcohol metabolizing enzymes- alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH2), and cytochrome P450 2E1 (CYP2E1) as well as proinflammatory cytokines (TNF-α, IL1β, IL6)
- Gene silencing approaches confirmed the regulatory role of lncRNA BACE1-AS in astrocytic amyloidosis and its interaction with mediators of alcohol metabolic pathways, leading to neuroinflammation and oxidative stress
- Ethanol-fed mice (4 weeks, ad libitum) demonstrated up-regulation of APP, Aβ1-42, 4-HNE and IL1β in the cortices compared to saline controls.
- This is the first report implicating the role of lncRNA BACE-AS in alcohol-mediated induction of astrocytic amyloidosis, leading, in turn, to neuroinflammation and oxidative stress, contributors of cognitive impairments.

Conclusion and Mechanism(s)

Alcohol use leads to upregulated expression of lncRNA BACE1-AS, neurotoxic amyloids, and dysregulated alcohol metabolism, ultimately leading to increased brain oxidative stress burden, neuroinflammation, and associated cognitive impairments.



Future Directions

- Role of each CNS cell type in the contribution of Alzheimer's like pathology in alcohol abuse.
  - Role of acute, chronic and binge drinking on neurological disorders.
- Role of alcohol metabolizing enzymes and end products in AD phenotype and inflammation.
  - Epigenetic modifications associated with alcohol abuse leading to cognitive disability.
  - Combinatorial effects with other drugs of abuse.

Acknowledgements

We are thankful to Dr. Saraswati Viswanathan, Division of Diabetes, Endocrinology, and Metabolism University of Nebraska Medical Center and VA for the *in vivo* samples. We are also thankful to Buch lab members for the support. Thank you to SURP and the UNMC MD-PHD Program for funding and support.