Alcohol-Induced Inflammasome Activation in the Intestine, Liver and Brain

PhD thesis

Dóra Lippai MD

Semmelweis University
Doctoral School of Clinical Medicine

Consultant: Gyöngyi Szabó MD, D.Sc

Official reviewers: Attila Zalatnai MD, Ph.D
Pál Demeter MD, Ph.D

Head of the Final Examination Committee: Ferenc Szalay MD, D.Sc
Members of the Final Examination Committee: János Banai MD, D.Sc
Gábor Veres MD, D.Sc
György Székely MD, Ph.D

Budapest
2016

To my Dearest Family

1. INTRODUCTION

1.1. Burden of alcohol-related diseases: Globally, harmful use of alcohol has been ranked among the top five risk factors for diseases and death. The amount of alcohol consumption, mainly containing ethanol, the drinking patterns and rarely the quality of alcohol are the major determinants of harmful outcome. Based on a 2010 report, Hungary was categorized among the countries with high consumption of alcohol per capita (≥12.5 liters). In Hungary in 2010 alcohol-attributable death was 13.3%.

The first line of treatment is prevention, cessation of alcohol use and proper nutrition. As a result of easy availability, addictive effect and harmful consequences of alcohol our effort should focus on finding a safe, effective and economically beneficial treatment for alcohol related diseases. In order to achieve a refined treatment, understanding the mechanism, cellular and subcellular changes as a result of alcohol consumption is important.

1.2. Effects of alcohol: Long term use of alcohol generally affects major organ systems. The effects include inflammatory changes and carcinogenesis.

During alcohol ingestion the proximal digestive system is exposed to high concentrations of alcohol, which leads to the most severe direct and pure effect of alcohol seen among the organs. Changes include bacterial overgrowth, barrier damage, PAMP translocation to the circulatory system, inflammation and carcinogenesis.

Alcohol and alcohol-induced P/DAMPs have easy access to the liver via the portal system. The major metabolism of alcohol occurs in the

liver, therefore the cellular effects of harmful derivates, including acetaldehyde is mainly expected in the liver of alcoholics. Consequences follow as chronic alcohol intake frequently induces fatty transformation and inflammation, resulting in steatohepatitis which might progress to cirrhosis and later hepatic cancer.

Alcohol can easily pass through the blood-brain barrier, including the endothelial sheet as well as the astrocyte wrap due to its small size and lipophilic property. Furthermore alcohol-induced P/DAMPs can indirectly or directly aggravate the harmful effects. Primarily alcohol alters behavior via changes in neurotransmission, further alcoholism leads to dementia, ataxia via neurodegeneration and possibly neuroinflammation.

1.3. Inflammasome: Alcohol-induced neuroinflammation and steatohepatitis is mediated by proinflammatory cytokines, including IL-1β. IL-1β production requires caspase-1 activation by inflammasomes, multiprotein complexes that are assembled in response to danger signals.

1.4. The significance of IL-1 signaling and inflammasome activation has not been evaluated in alcoholic intestinal, liver and brain injury.

2. OBJECTIVES

2.1. We aimed to evaluate the inflammasome activation in the proximal intestine of an animal model of alcoholism.

2.2. We aimed to evaluate the inflammasome activation in the cerebellum of an animal model of alcoholism.

3. METHODS

3.1. WT and TLR4-, NLRP3-, and ASC-KO mice received an ethanol-containing or isocaloric control diet for 5 weeks, and some received the rIL-1Ra, anakinra, or saline treatment ip. Animals on acute ethanol-regime received 5g/kg 50% (v/v) ethanol diluted in water or an equal amount of water via oral gavage for three consecutive days. Some anesthetized mice received 30μl intracranial injection with recombinant mouse IL-1β or equal amount of saline solution.

3.2. Serum alcohol and endotoxin concentration was measured by alcohol analyzer and LAL assay.

3.3. Inflammasome and NF-κB activation, Reg3b, PRRs, proinflammatory cytokines, HMGB1 and endotoxin were measured in murine proximal intestine, cerebellum and cerebrum using qPCR, ELISA, WB, enzyme-activity assay, IP and LAL assay.

3.4. Kruskall-Wallis nonparametric test was used to analyze the data.

4. RESULTS

4.1. Proximal intestine

4.1.1. Mice receiving either one-time alcohol gavage or 5 weeks of alcohol feeding have significantly higher blood alcohol content than their appropriate controls.

4.1.2. Mice receiving either three days alcohol gavage or 5 weeks of alcohol feeding have significantly higher serum-endotoxin

levels than their appropriate controls. Twelve hours after alcohol gavage, no difference of serum-endotoxin level is found between alcohol gavage and control groups.

4.1.3. Both mRNA as well as protein level of Reg3b is significantly higher in the small intestine of mice receiving alcohol gavage than their controls. In contrast, the expression of Reg3b in the small intestine of chronic alcohol-fed mice is significantly lower both at mRNA as well as protein level compared to controls.

4.1.4. Both alcohol gavage and chronic alcohol feeding results in significantly higher NF-κB activation and DNA binding in proximal small intestine of mice compared to controls. The magnitude of increase is significantly greater in mice with chronic compared to acute binge alcohol administration

4.1.5. TNFα mRNA levels are significantly higher in the proximal small intestine of mice after both acute binge and chronic alcohol feeding compared to controls. However, murine proximal small intestinal TNFα protein levels are significantly higher after chronic alcohol feeding, no difference of TNFα protein level is found between alcohol gavage and control groups.

4.1.6. No difference of murine proximal intestinal pro-IL-1β mRNA or IL-1β protein level is found between either alcohol gavage or chronic alcohol-feeding and appropriate control groups.

4.1.7. No difference of murine proximal small intestinal NLRP3, NLRP6, ASC and pro-caspase-1 mRNA is found between either alcohol gavage or chronic alcohol-feeding and appropriate control groups.

4.2. Cerebellum

4.2.1. Chronic alcohol feeding results in significantly higher NF-κB activation in cerebella of mice compared to controls.

4.2.2. Both TNFα mRNA and protein levels are significantly higher in the cerebella of chronic alcohol-fed than control mice.

4.2.3. Pro-IL-1β mRNA as well as protein levels are significantly higher in the cerebella of mice after chronic alcohol feeding compared to controls. Similar to cerebellar IL-1β protein levels detected by specific ELISA, Western blot result shows significantly higher mature IL-1β protein levels in the cerebellum of chronic alcohol-fed compared to control mice. Both pro-IL-1β mRNA and IL-1β protein are significantly higher in the cerebral cortex of chronic alcohol-fed compared to control mice. The magnitude of IL-1β protein increase does not differ between murine cerebellar and cerebral cortex upon chronic alcohol feeding.

4.2.4. Murine cerebellar NLRP1, NLRP3, ASC and pro-caspase-1 mRNAs are significantly higher after chronic alcohol-feeding compared to controls. Similarly the levels of cerebral NLRP1, NLRP3, ASC and pro-caspase-1 mRNAs are significantly higher in chronic alcohol-fed compared to control mice.

4.2.5. Caspase-1 activity is significantly higher in murine cerebellum and cerebrum after chronic alcohol feeding compared to controls. Pro-caspase-1 and caspase-1 p10 are significantly higher in murine cerebellum after chronic alcohol feeding compared to controls.

4.2.6. Mice receiving 5 weeks of alcohol feeding have significantly higher blood alcohol content than their appropriate controls regardless of their genotype (WT; TLR4-KO; NLRP3-KO; ASC-KO). No statistically significant difference of blood alcohol content of mice is found among different genotypes (WT vs TLR4-KO; WT vs NLRP3-KO and ASC-KO) after 5 weeks of alcohol feeding.

4.2.7. Cerebellar IL-1β protein level is significantly higher after chronic alcohol feeding in WT mice whereas no significant difference of cerebellar IL-1β protein level is found between alcohol-fed NLRP3-KO or ASC-KO and their appropriate control mice. Cerebellar mature IL-1β protein level is significantly lower in chronic alcohol-fed NLRP3-KO compared to WT mice.

4.2.8. No difference of cerebellar caspase-1 activity is found between chronic alcohol-fed NLRP3-KO or ASC-KO and their appropriate control mice

4.2.9. Murine cerebellar Toll-like receptors, TLR2, TLR4, TLR9 and receptor for advanced glycation end products (RAGE) mRNAs are significantly higher after chronic alcohol-feeding compared to controls.

4.2.10. No significant difference of cerebellar TNFα protein level is found between chronic alcohol-fed TLR4-KO and isocaloric control mice. Furthermore, cerebellar TNFα protein level is significantly lower in chronic alcohol-fed TLR4-KO compared to WT mice.

4.2.11. The levels of cerebellar pro-IL-1β mRNA and IL-1β protein are significantly higher in chronic alcohol-fed TLR4-KO compared to isocaloric control mice. The levels of cerebellar pro-IL-1β mRNA and IL-1β protein are significantly lower in either isocaloric or chronic alcohol-fed TLR4-KO compared to WT mice.

4.2.12. Cerebellar TLR4 is not detectable in TLR4-KO mice and therefore is significantly lower than WT controls receiving either chronic alcohol or isocaloric diet. The level of cerebellar NLRP1, NLRP3, ASC and pro-caspase-1 mRNA is significantly higher in chronic alcohol-fed WT or TLR4-KO compared to isocaloric control mice. The level of cerebellar NLRP1, NLRP3, ASC and pro-caspase-1 mRNA is significantly lower in either isocaloric or chronic alcohol-fed TLR4-KO compared to WT mice.

4.2.13. Caspase-1 activity is significantly higher in TLR4-KO murine cerebellum after chronic alcohol feeding compared to controls. No significant difference of cerebellar caspase-1 activity is found between chronic alcohol-fed WT and TLR4-KO mice.

4.2.14. No difference of cerebellar endotoxin level is found between chronic alcohol-fed and control mice.

4.2.15. Cerebellar HMGB1 mRNA is significantly higher in chronic alcohol-fed compared to control mice. No significant difference of cerebellar total HMGB1 protein is found between chronic alcohol-fed and control mice. Using immunoprecipitation, both acetylated- and phosphorylated-HMGB1 levels are significantly higher in the cerebellum of chronic alcohol-fed compared to control mice.

4.2.16. No difference of cerebellar interleukin-1 receptor (IL-1R) is found between chronic alcohol-fed and control mice. Mice receiving 5 weeks of alcohol feeding have significantly higher endogenous IL-1R antagonist levels in the cerebellum compared to controls.

4.2.17. No significant difference of cerebellar TNFα mRNA is found between chronic alcohol-fed and isocaloric control mice after recombinant interleukin-1 receptor antagonist (rIL-1Ra) treatment. In contrast cerebellar TNFα protein is significantly higher in chronic alcohol-fed rIL-1Ra compared to appropriate isocaloric control mice. Both cerebellar TNFα mRNA and protein levels are significantly lower in rIL-1Ra compared to saline treated mice after chronic alcohol feeding.

4.2.18. No significant difference of either cerebellar pro-IL-1β mRNA or IL-1β protein is found between chronic alcohol-fed recombinant interleukin-1 receptor antagonist (rIL-1Ra) and appropriate isocaloric control mice. The background of cerebellar IL-1β protein level is significantly higher in rIL-1Ra compared to saline treated mice receiving isocaloric diet.

4.2.19. No significant difference of cerebellar receptorial (TLR2, TLR4, TLR9), inflammasome (NLRP1, NLRP3, ASC, pro-caspase-1), IL-1R or endogenous IL-1Ra mRNA is found between chronic alcohol-fed rIL-1Ra and isocaloric control mice.

4.2.20. Caspase-1 activity is significantly higher in chronic alcohol-fed rIL-1Ra treated compared to appropriate isocaloric control

mice. Cerebellar caspase-1 activity is significantly lower in rIL-1Ra treated compared to saline treated mice after chronic alcohol feeding.

4.2.21. No significant difference of cerebellar acetylated-HMGB1 is found between chronic alcohol-fed rIL-1Ra treated and appropriate isocaloric control mice.

4.2.22. Cerebellar TNFα and pro-IL-1β mRNA as well as TNFα protein are significantly higher in mice receiving intracranial recombinant IL-1β injection compared to saline treated controls.

CONCLUSIONS

4.3. Both acute and chronic alcohol intake impair intestinal barrier function; the increased serum endotoxin after acute alcohol binge is transient.

4.4. Increased serum endotoxin after chronic alcohol administration is associated with reduced Reg3b whereas acute alcohol administration induces Reg3b production.

4.5. Chronic alcohol intake induces inflammation in the gut-liver-brain axis, including NF-κB activation and TNFα production.

4.6. Chronic alcohol administration up-regulates and activates the NLRP3/ASC inflammasome leading to caspase-1 activation and IL-1β production in the liver and cerebellum in contrast to the proximal small intestine.

4.7. Chronic alcohol intake increases the levels of phosphorylated and acetylated forms of HMGB1 in the cerebellum.

4.8. Disruption of IL-1/IL-1R signaling by rIL-1Ra prevents alcohol-induced inflammasome activation, neuroinflammation and

steatohepatitis by inhibiting inflammasome activation, IL-1β and TNFα production.

4.9. IL-1β amplifies neuroinflammation and steatohepatitis.

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6. ACKNOWLEDGEMENTS

I would like to thank my PhD supervisor, **Professor Gyongyi Szabo**, for supporting me during these past four years. Professor Szabo was always supportive, enthusiastic and energetic and has given me the freedom to pursue various projects without objection and has provided insightful discussions about the research.

I would like to thank **Professor Zsolt Tulassay**, a great advisor, manager and mentor, who has introduced me to Professor Szabo in order to have a fruitful relationship.

I would like to thank members of Dr Szabo’s laboratory, **Shashi Bala, Timea Csak, Arijeet Gattu, Jan Petrasek, Karen Kodys, Donna Catalano, Ivan Levin, Anna Cerny, Terence Bukong,**

Shuye Zhang and **Matthew Barrieu** for the help and critical atmosphere which was useful for the development of the study.

I would like to appreciate our collaborator, **Professor Kurt-Jones** for her help.

The core of this research project was executed at the **University of Massachusetts Medical School**. This project was supported by **U.S. National Institutes of Health** grants AA017729 and AA011576.