The In Vivo Effects of Alcohol in Lung and Liver are at Least Partially Mediated through the Alpha 4 Nicotinic Acetylcholine Receptor

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Abstract

Rationale: Chronic alcohol abuse is a major risk factor for the development of acute lung injury, with 40% of annual cases in the U.S. linked to this disorder. Alcohol is not only associated with increased incidence of acute lung injury, but also increased mortality. Several mechanisms by which alcohol abuse renders the host susceptible to acute lung injury remain poorly defined. We have reported that α4 nicotinic acetylcholine receptors (α4 nAChRs) may serve as potential sensors for alcohol in lung fibroblasts; however, we have not tested their role in vivo.

Methods: To test the role of α4 nAChRs in mediating alcohol-related events in vivo, we generated α4 KO (α4 knockout) (KO) animals in C57Bl/10 using Csnpr/Cas technology. Wildtype (WT) and α4 KO (α4 KO) animals were used to harvest primary lung fibroblasts for study in vitro. In vivo experiments included exposure to Lieber-DeCarli isocaloric or Maltose-Dextrin control diet for 6 weeks.

Results: Having ensured that the α4 KO animals indeed lacked the α4 nAChRs, we isolated primary lung fibroblasts and evaluated their expression of the matrix glycoprotein fibronectin after exposure to nicotine (50 μg/ml) or alcohol (60 μM). As expected, nicotine induced fibronectin expression independent of the presence or absence of α4 nAChRs. In contrast, alcohol induced fibronectin mRNA expression in primary lung fibroblasts harvested from WT animals, but not from α4 KO animals. We then engaged in vivo studies designed to examine the expression of specific genes in whole lung and liver; including the cysteine transporter Slc7a11 (which controls redox state), the pro-inflammatory cytokine TnFα (which has been implicated in alcohol-induced lung injury), and the protease inhibitor PA-1 (which also appears involved in alcohol-related injury to lung and liver). No overt structural abnormalities were detected in the α4 KO animals. After 6 weeks of control or alcohol diets, lungs and livers were harvested and processed for mRNA evaluation. WT lungs and livers showed significant induction of all three mRNAs when exposed to alcohol, whereas the α4 KO animals showed little to no induction. Liver histology also showed evidence of increased steatosis in WT animals when compared to the α4 KO animals.

Conclusions

1. α4 deficient animals do not demonstrate obvious pulmonary or liver structural abnormalities (not shown).
2. In primary lung fibroblasts, alcohol stimulates the expression of fibronectin via α4 nAChRs, while nicotine acts via other nAChRs (likely α7) (Fig. 2).
3. Alcohol not only acts via α4 nAChRs, it also enhances its expression (Fig. 2).
4. In vivo, alcohol stimulates the expression of several inflammatory markers and a cysteine transporter in lung via α4 nAChRs (Fig. 3).
5. By affecting α4 nAChRs, alcohol promotes inflammation in liver as highlighted by increased liver transaminases (Fig. 4), increased expression of inflammatory markers (Fig. 5), fat accumulation (Fig. 6).

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