PRECLINICAL EVALUATION OF SODIUM BUTYRATE'S POTENTIAL TO REDUCE ALCOHOL CONSUMPTION: A DOSE ESCALATION STUDY IN C57BL MICE IN ANTIBIOTIC-ENHANCED BINGE-LIKE DRINKING MODEL

G. Havton, A. Tai, S. Vasisht, A. Batdorj, K. Frost, D.L. Davies, and L. Asatryan

School of Pharmacy, University of Southern California, Los Angeles, CA, 90033

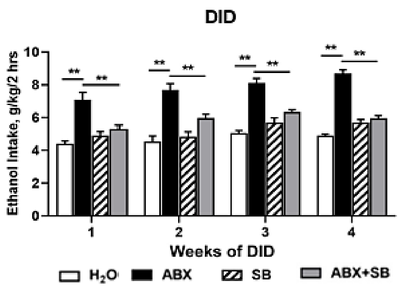
USC Mann
Alfred E. Mann School of Pharmacy and Pharmaceutical Sciences

INTRODUCTION

- Emerging evidence supports the link between changes in gut microbiome and development of neuropathologies, including alcohol use disorder (AUD)[1-3].
- Chronic alcohol abuse causes changes in the levels of gut bacteria-derived metabolites, such as short-chain fatty acids (SCFAs). An essential SSCFA butyrate has been implicated in anti-inflammatory responses through modulation of peripheral immune system function, stimulation of the vagus nerve, and endocrine signaling[4]. Our previous work with the use of non-absorbable antibiotic cocktail (ABX) to induce dysbiosis, caused dramatic reduction in butyrate-producing bacterial populations and this was inversely correlated with ethanol consumption levels in mice[3].
- In the follow-up study, *ad libitum* supplementation of C57BL/6J male mice with 8 mg/ml sodium butyrate (SB) reduced ethanol intake and neuroinflammatory response in antibiotic (ABX)-enhanced voluntary binge-like alcohol consumption model[5] (Fig. 1).

AIM: To further evaluate the pre-clinical potential of SB, we have set a dose-escalation study and tested the effects of 20 and 50 mg/ml SB (respectively SB-20 and SB-50) in the ABX-enhanced binge-like alcohol consumption model.

Fig 1. Effects of Sodium Buyrate (SB) Supplementation on ABX-Induced Increased in Ethanol Intake



- ABX-induced an increase in ethanol intake which was prevented by SB supplementation in DID/binge-like drinking model. **p,0.01.

REFERENCES

1. Dubinkina, V.B., A.V., Odintsova, V.Y, et al & Govorun VM. (2017) Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. Microbiome, 5(1), 141.
2. Peacock, A., Leung, J., Larnery, S., et al., & Degenhardt, L. (2018).Global statistics on alcohol, tobacco, and illicit drug use: 2017 status report. Addiction, 113(10) 1905e1926.
3. Reyes, R. E. N., Al Omran, A. J., Davies, D. L., & Asatryan, L. (2020). Antibiotic-induced disruption of commensal microbiome linked to increases in binge-like ethanol consumption behavior. Brain research, 1747, 147067.
4. Stilling, R. M., van de Wouw, M., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2016). The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis?. Neurochemistry international, 99, 110–132.
5. Reyes, R. E., Gao, L., Zhang, Z., Davies, D. L., & Asatryan, L. (2022). Supplementation with sodium butyrate protects against antibiotic-induced increases in ethanol consumption behavior in mice. Alcohol (Fayetteville, N.Y.), 100, 1–9.

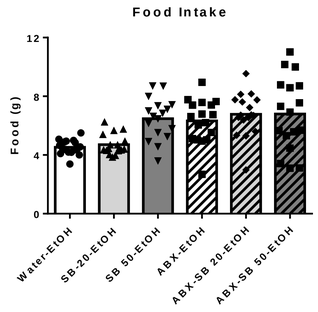
ACKNOWLEDGEMENT

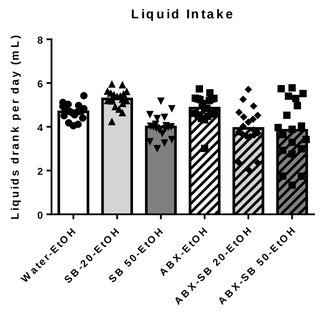
We would like to thank our graduate, undergraduate, and high school students for all taking part in this research, as it is a group effort to keep the project moving.

Support: Titus Fund Research Award (USC; to LA), Rose Hills Foundation Innovator Award (USC; to LA), USC School of Pharmacy and USC Good Neighbors

RESULTS

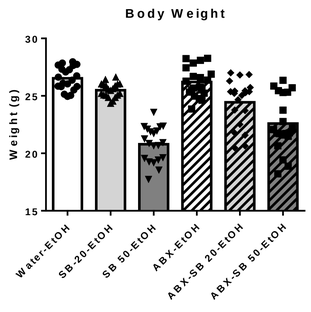
Fig. 2. Effects of Sodium Butyrate (SB) Concentrations on the Overall Food and Liquid (non ethanol) Intake

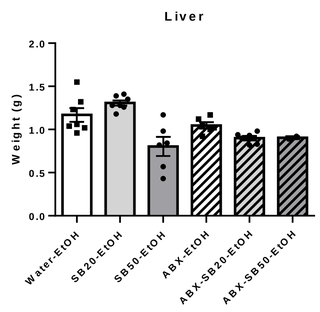


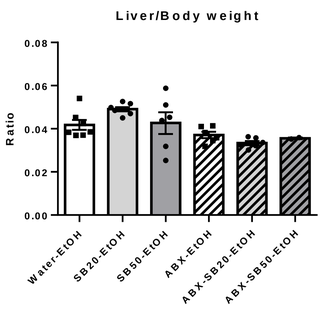


- SB 20 did not cause changes in food intake but significantly increased liquid intake (p< 0.001 vs water group) for the duration of the study.
- SB50 significantly increased food intake (p<0.001) and significantly reduced liquid intake (p<0.001) compared to the water group.
- SB 20 and SB 50 did not affect food intake but significantly reduced liquid intake (p<0.005) when applied with ABX

Fig. 3. Effects of SB Concentrations on Bodily Parameters

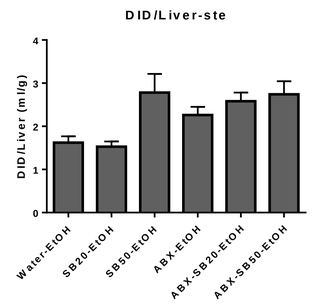


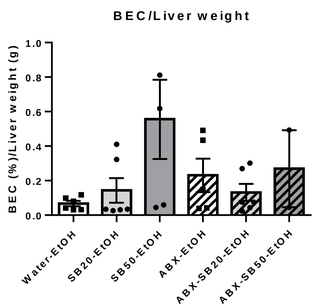




- Body and liver weights were significantly lower in the SB 50 group (p<0.001 vs water group). Body and liver weights were also significantly lower (p<0.001) when supplemented with ABX for both SB concentrations.

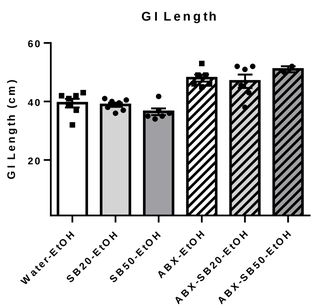
Fig. 4. Effects of SB Concentrations on Ethanol Intake and Blood Ethanol Concentrations

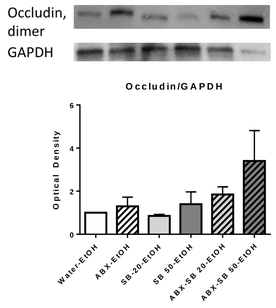


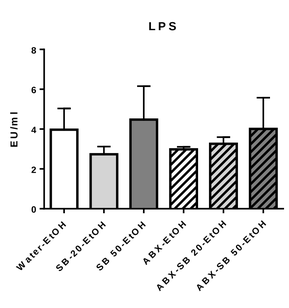


- SB 50 significantly increased ethanol intake in the DID procedure with and without ABX treatment (p<0.05 for both comparisons).
- Blood ethanol concentrations (BECs) were also higher with SB 50 treatment.

Fig. 5. Effects of SB Concentrations on Intestinal Parameters







- Supplementation with SB20 did not affect the GI length or intestinal occludin (dimer) levels. In contrast, SB50 reduced the GI length, caused an increase in occludin expression in the absence and presence of ABX. In addition, there was a trend to increase the serum LPS levels with SB50 supplementation.

METHODS

Mice and Treatments: 6-8 week old C57BL/6J mice were single-housed on a 12 hr light/dark cycle and received food ad libitum. Mice were then assigned to four treatment groups, provided ad libitum in drinking water:H2O (n=7), antibiotic cocktail (ABX) (n=7), sodium butyrate 20mg/ml (SB20; n=7), SB 50 mg/ml (n=7), ABX + SB 20mg/ml (n=7), ABX + SB 50mg/ml (n=7). Mice were pre-treated for 2 weeks prior to the start of the drinking-in-the-dark (DID) procedure. Mice were subjected to DID for. Non-absorbable broad- spectrum ABX cocktail included: bacitracin, neomycin, vancomycin, and pimaricin (antifungal). Data presented as Mean ± SEM. One-way ANOVA and a t-test for individual group comparisons were performed using Graph Prism

Ethanol Consumption Model (DID): Mice were given 20% EtOH three hours into the dark cycle for two hours to replace their treatment bottle Monday-Friday for 4 weeks. Ethanol intake in DID was calculated as g (pure ethanol)/kg (body weight).

Measurements: Body weights (in grams), food (in grams) and fluid (in mL) intakes were measured every other days. After necropsies, liver weights (in grams, intestinal lengths (cm) were measured, blood collected for BEC measurement (Sigma kit). Serum LPS was also measured using Pierce Chromogenic Endotoxin Quant kit. Western immunoblotting was performed per procedures in place in lab. Occludin antibody was from Abcam.

Data Analysis: Data were analyzed using GraphPad Prism and are presented as Mean ± SEM. One-way ANOVA was performed for group comparisons. Western blot analysis was performed using Image J software.

DISCUSSION

Effects of SB 20 supplementation:

- SB-20 alone did not cause changes in food intake but significantly reduced liquid intake when applied with ABX.
- Body and liver weights were not changed with SB 20 supplementation.
- SB 20 was not able to reduce ABX-enhanced ethanol intake. This may have been related to some smell or taste aversion to SB which is apparent during the reduced liquid intake in non-DID period.
- SB 20 supplementation did not affect intestinal parameters as measured through the GI length, tight junction occludin level (dimer at ~105 kDa), and serum endotoxin LPS level.

Effects of SB 50 supplementation:

- SB 50 significantly increased the food and reduced liquid intakes in the absence and presence of ABX treatment. Body and liver weights were significantly reduced with SB 50
- SB 50 increased ethanol intake and BEC levels. Reduced weight parameters as well as increased liquid intake during the DID session may be associated with reduced liquid intake during the non-DID period due to smell or taste-induced aversion.
- There was shorter GI length, increased occludin dimer expression and a trend towards higher serum LPS levels with SB 50 supplementation.

CONCLUSIONS

- Overall, these findings suggest that supplementation with SB-20 but not SB-50 is tolerated by mice.
- Beneficial effects of SB on ethanol intake may be masked due to smell/taste aversion at high SB concentrations(both 20 and 50 mg/ml) provided *ad libitum*.
- Other routes of administration such as intraperitoneal injections or combining butyrate with food may be more suitable approaches to avoid SB smell/taste aversion in mice and suffice in better evaluation of its preclinical potential for AUD.
- Additional analyses in on the way to detect any adverse effects on the intestinal, liver and brain functions.