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Probiotics attenuate alcohol-induced muscle mechanical hyperalgesia: Preliminary observations

Paul G Green[1,2,3], Pedro Alvarez[1,3], Jon D Levine[1,3,4]

Abstract

Alcohol use disorder (AUD) is a major health problem that causes millions of deaths annually world-wide. AUD is considered to be a chronic pain disorder, that is exacerbated by alcohol withdrawal, contributing to a high (~80%) relapse rate. Chronic alcohol consumption has a marked impact on the gut microbiome, recognized to have a significant effect on chronic pain. We tested the hypothesis that modulating gut microbiota through feeding rats with probiotics can attenuate alcohol-induced muscle mechanical hyperalgesia. To test this hypothesis, rats were fed alcohol (6.5%, 4 days on 3 days off) for 3 weeks, which induced skeletal muscle mechanical hyperalgesia. Following alcohol feeding, at which time nociceptive thresholds were ~37% below pre-alcohol levels, rats received probiotics in their drinking water, either *Lactobacillus Rhamnosus* GG (Culturelle) or De Simone Formulation (a mixture of 8 bacterial species) for 8 days; control rats received plain water to drink. When muscle mechanical nociceptive threshold was evaluated 1 day after beginning probiotic feeding, nociceptive thresholds were significantly higher than rats not receiving probiotics. Mechanical nociceptive thresholds continued to increase during probiotic feeding, with thresholds approaching pre-alcohol levels 5 days after starting probiotics; nociceptive threshold in rats not receiving probiotics remained low. After probiotics were removed from the drinking water, nociceptive thresholds gradually decreased in these two groups, although they remained higher than the group not treated with probiotic (21 days after ending alcohol feeding). These observations suggest that modification of gut microbiota through probiotic feeding has a marked effect on chronic alcohol-induced muscle mechanical hyperalgesia. Our results suggest that administration of probiotics to individuals with AUD may reduce pain associated with alcohol consumption and withdrawal, and may be a novel therapeutic intervention to reduce the high rate of relapse seen in individuals with AUD attempting to abstain from alcohol.

Keywords

Ethanol, myalgia, muscle pain, nociceptors, probiotics, microbiome

Introduction

Chronic alcohol consumption can lead to alcohol use disorder (AUD), which is highly comorbid with chronic pain,[1–3] with AUD itself being considered a chronic pain disorder.[4] Approximately 30% of the U.S. population meets AUD criteria on a lifetime basis,[5] and AUD has substantially increased world-wide in the last three decades.[6] In addition to being a risk factor for the development of chronic pain,[2] individuals with AUD undergoing acute alcohol withdrawal exhibit greater pain sensitivity,[7–9] which in turn can lead to relapse in recovering from AUD.[2] Unfortunately, there are few effective therapies to treat AUD-mediated pain and individuals

[1]Departments of Oral and Maxillofacial Surgery, University of California San Francisco, San Francisco, CA, USA
[2]Departments of Preventative and Restorative Dental Sciences, University of California San Francisco, San Francisco, CA, USA
[3]Division of Neuroscience, University of California San Francisco, San Francisco, CA, USA
[4]Departments of Medicine, University of California San Francisco, San Francisco, CA, USA

Corresponding Author:
Jon D Levine, University of California San Francisco, San Francisco, CA 94143, USA.
Email: jon.levine@ucsf.edu

undergoing AUD treatment programs have a very high, 70–85%, relapse rate.[10,11] Therefore, new therapeutic approaches are needed to treat AUD, in particular AUD-associated pain.

It is well established that chronic alcohol consumption has a marked impact on the gastrointestinal (gut) microbiome, as well as increasing gut permeability.[12,13] Given the recent appreciation of the ability of gut microbiota to modulate chronic pain,[14–17] and that gut microbial diversity is markedly affected by alcohol consumption,[18–20] we hypothesized that feeding probiotics, to ameliorate alcohol-induced dysbiosis, reduces alcohol-induced muscle hyperalgesia.

Methods

Animals

Adult (260–300 g) male Sprague Dawley rats were obtained from Charles River (Hollister, CA), and were housed in the Laboratory Animal Resource Center of the University of California, San Francisco, under a 12 h light/dark cycle (lights on 7 a.m.–7 p.m.) and environmentally controlled conditions; ambient room temperature (21°–23°C), with food and water available ad libitum. Their care and use in experiments conformed to National Institutes of Health guidelines and measures were taken to minimize pain and discomfort. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

Chronic alcohol consumption

Rats were fed a Lieber-DeCarli liquid diet (ethanol rat diet # 710260, Dyets Inc, Bethlehem, PA),[21] containing 6.5% ethanol; the liquid diet contains all necessary nutrients to replace rat chow. This is the standard ethanol-containing liquid diet for rats from this manufacturer, and this concentration of ethanol in liquid diet has been used by us[22–27] and others.[28,29] We employed our 'binge drinking' model,[26] in which rats were exposed to weekly cycles of 4 days on 6.5% ethanol-containing liquid diet and 3 days of standard laboratory rat chow, for 3 weeks (i.e. ethanol diet on days 0–4, 7–11 and 14–18).

Administration of probiotics

On day 20, two days after the alcohol feeding period ended, rats were divided into 3 groups: the control group received drinking water containing only tap water, another group received *Lactobacillus rhamnosus* GG in their drinking water (10 billion CFU/150 ml *L. rhamnosus*, Culturelle®, Amerifit, Inc, Cromwell, CT), and the third group received De Simone Formulation (DSF) in their drinking water (112.5 billion CFU/150 ml mixture of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subspecies bulgaricus, Bifidobacterium breve, *B. longum*, *B. infantis*, and *Streptococcus salivarius* subspecies thermophilus, DSF, VSL Pharmaceuticals, Inc, Towson, MD). Drinking water containing probiotics was made fresh each day. Water or probiotic-containing water was provided to rats ad libitum for 8 days (day 20–28).

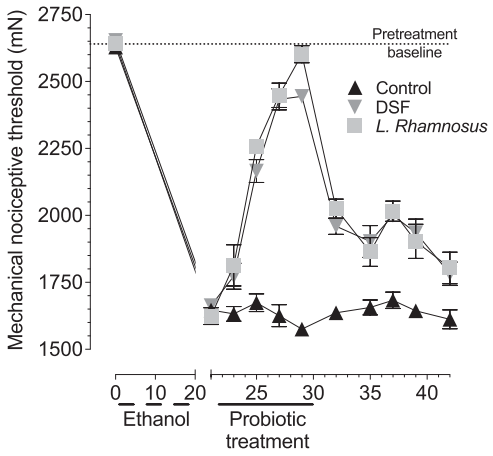

Figure 1. Administration of DSF or *L. rhamnosus* attenuates chronic alcohol-induced muscle mechanical hyperalgesia. Time course of muscle mechanical hyperalgesia induced by chronic alcohol consumption, shows that rats receiving three cycles of ethanol diet (4 days on, 3 days off) produces robust mechanical hyperalgesia by day 21 (nociceptive threshold was ~37% lower compared to pre-alcohol feeding). After alcohol feeding, rats were divided into three groups, receiving water alone (control), or DSF and *L. rhamnosus* in their drinking water, beginning on day 22. The two-way repeated measures ANOVA showed a significant group × time interaction ($F_{20,150} = 33.83$; $P < 0.0001$) and a significant main effect of group ($F_{2,15} = 66.16$; $P < 0.0001$), indicating that the groups differed significantly in both time course and magnitude. Tukey's multiple comparison showed significant differences between control and DSF, and between control and *L. rhamnosus*, for all time points, beginning on day 23 (1 day after probiotic feeding), and continuing through last time point measured (day 42).

Mechanical nociceptive threshold in muscle

Mechanical nociceptive threshold in the gastrocnemius muscle was quantified using a Chatillon digital force transducer (model DFI2, Amtek Inc, Largo, FL).[30] Rats were placed in cylindrical acrylic restrainers designed to minimize restraint stress and allow for hind leg extension from lateral ports. To acclimatize rats to the testing procedure, they were trained in the nociceptive testing protocol daily for 3 days prior to starting experiments. On the day of the experiment, rats were placed in a restrainer for 30 min before experimental manipulations. To measure nociceptive threshold, the hind limb was gently extended, and a 6 mm diameter probe attached to the force transducer was applied to the gastrocnemius muscle to deliver an increasing compression force. The nociceptive threshold was defined as the force, in

Newtons, at which the rat withdrew its hind leg. Baseline withdrawal threshold was defined as the mean of 3 readings taken at 5 min intervals. All behavioral testing was conducted by the same individual, between 10 a.m. and 4 p.m., who was blind to treatment condition.

Statistical analyses

Group data are expressed as mean ± SEM of *n* independent observations. Statistical analyses of experimental data were conducted using repeated-measures two-way analysis of variance (ANOVA), using Prism 9 (GraphPad Software, San Diego, CA). Where ANOVA showed a significant main difference between treatments groups, Tukey's multi-comparison post hoc test was used to determine the pair of treatment groups that were different. The accepted level for statistical significance was $P < 0.05$.

Results

Probiotics markedly attenuated alcohol-induced hyperalgesia

Mechanical nociceptive threshold decreased ~37% following the 3 weeks of 4 days on/3 days off alcohol feeding protocol, demonstrating robust alcohol-induced hyperalgesia. On day 21 (after alcohol feeding), rats' drinking water contained either plain tap water (control group), DSF or *L. rhamnosus* (Figure 1). When mechanical nociceptive threshold was evaluated 1 day later, there was a small, but significant, reversal of hyperalgesia in the probiotic-fed rats compared to control group (Control vs DSF $P = 0.028$; Control vs *L.* rhamnosus $P = 0.003$). During the 8 days of probiotic feeding (day 20–28), nociceptive threshold was markedly increased (days 25, 27, and 29, $P < 0.0001$ for both DSF and *L.* rhamnosus compared to control), reaching pre-alcohol baseline nociceptive threshold. Following cessation of probiotic feeding, nociceptive threshold in the probiotic groups decreased over time, although it was still significantly higher than the control group by day 42 (Control vs DSF $P = 0.006$; Control vs *L.* rhamnosus $P = 0.002$).

Discussion

In this study, we observed that alcohol-induced mechanical hyperalgesia was markedly attenuated in rats receiving probiotics, *L. rhamnosus* or DSF, suggesting that modification of gut microbiota has a pronounced effect on chronic alcohol-induced muscle mechanical hyperalgesia. A relationship between gut microbiota and pain has previously been suggested, with preclinical and clinical studies showing that visceral hyperalgesia and abdominal pain are associated with gut dysbiosis,[31–34] and that visceral pain can be attenuated by administration of probiotics, including DSF[35–37] and *L.* rhamnosus.[37–39] However, there are very few published studies that have examined whether associations between gut dysbiosis and pain syndromes occur for pain outside the abdominal region. It has been reported that pain syndromes, such as back pain,[40] as well as systemic pain conditions, such as chronic widespread pain[41] and fibromyalgia,[42,43] and chemotherapy-induced painful peripheral neuropathy[44] are affected by the gut microbiome. However, to the best of our knowledge our current study is the first to suggest that modifying the gut microbiome can have a marked effect at reversing alcohol-induced muscle mechanical hyperalgesia.

While our finding that alcohol-induced hyperalgesia is reversed by consumption of probiotics, the mechanism underlying this effect is yet to be determined. In individuals with AUD, as well as in preclinical models of chronic alcohol feeding, there are significant changes in the gut microbiome.[45–48] However, it is not possible at this point to determine which bacterial species may contribute to muscle hyperalgesia given the gut's vast and diverse microbial composition of 500–1000 species of bacteria,[49] many of which are up- or down-regulated by chronic alcohol consumption.[12,45,47,50,51] Furthermore, in addition to bacteria, there are immense populations of fungi, viruses, archaea, and helminths in the gut[52] that may also be affected by alcohol consumption in humans and contribute to changes in nociceptive threshold. However, there is strong evidence from meta-analyses that bacteria alone affect pain,[51] and clinical studies showing that the severity of fibromyalgia pain is correlated with gut *Bacteroides* spp. levels.[53] Therefore, our observation that DSF and *L.* rhamnosus reverses alcohol-induced muscle mechanical hyperalgesia may be due to the probiotics rebalancing the gut microbiota in rats chronically consuming ethanol; both DSF and *L.* rhamnosus have been shown to normalize gut dysbiosis produced by a number of factors, including alcohol consumption.[46,50,54–58]

In addition to causing gut dysbiosis, chronic alcohol consumption disrupts intestinal epithelial tight junctions and the mucosal barrier, thereby increasing gut permeability.[12,13,59,60] Increased permeability allows for translocation of microbes and bacterial products from the intestine to the systemic circulation[13]; bacteria can directly activate nociceptors via their constitutive elements and products,[14,61,62] such as bioactive lipids, and short chain fatty acids, such as butyrate,[49] that increases nociceptor sensitivity,[63] as well as lipopolysaccharide, the ligand for toll-like receptor 4 that markedly increases nociceptor sensitization.[64] Therefore, the reduction of alcohol-induced muscle hyperalgesia produced by DSF and *L.* rhamnosus may be related to their ability to reverse alcohol-induced increased gut permeability. Administration of both DSF[65] and *L.* rhamnosus treatment[46,66] has been shown to attenuate alcohol-induced increased gut permeability to decrease the translocation of lipopolysaccharide and other bacterial products into the circulation.[46,65] Additional studies will be needed to determine which of the 8 bacterial species in DSF are able to reverse alcohol-induced muscle hyperalgesia.

Individuals with AUD undergoing alcohol withdrawal experience pain and hyperalgesia, which is believed to contribute to relapse drinking.[8,67,68] In addition, reduction in pain during treatment for AUD is associated with lower risk for relapse.[69] With AUD causing 2.3 million deaths per year,[70] and with a relapse rate of ~80% one year post-withdrawal,[10,11,71] there is an urgent need for new therapies to treat AUD. Our results suggest that consumption of probiotics may reduce pain in individuals with AUD, which could suppress their tendency to relapse. However, our study shows that relatively short-term feeding of probiotics does not result in persistent protection from alcohol-induced hyperalgesia, following cessation of probiotic feeding. Whether a prolonged feeding could result in persistent protection beyond probiotic feeding is an open question. There is evidence that probiotic microbes persist for only a few weeks after consumption.[72,73] However, establishment and persistence of probiotics may be dependent on strain and diet.[74] Thus, from a therapeutic standpoint, probiotics may need to be consumed chronically to provide ongoing antihyperalgesia protection. Additional studies will be needed to determine whether specific probiotic species may confer long-lasting anti-hyperalgesic effects in alcohol and other neuropathies, and to determine the mechanisms of action, as well as clinical studies to evaluate the potential efficacy in patients with alcohol-induced pain.

Declaration of Conflicting Interests

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ORCID iD

Paul G Green https://orcid.org/0000-0001-7648-6826

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