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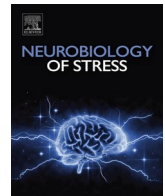Estey, David
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A reverse translational study of PPAR-α agonist efficacy in human and rodent models relevant to alcohol use disorder

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ABSTRACT

Alcohol Use Disorder (AUD) is a chronic relapsing disorder affecting an estimated 283 million individuals worldwide, with substantial health and economic consequences. Peroxisome proliferator-activated receptors (PPARs), particularly PPAR-α and PPAR-γ, have shown promise in preclinical studies as potential therapeutic targets for AUD. In this human laboratory study, we aimed to translate preclinical findings on the PPAR-α agonist fenofibrate to a human population with current AUD. We hypothesized that, relative to placebo, fenofibrate at the highest FDA-approved dose of 145 mg/d would attenuate responsiveness to *in vivo* alcohol cues in the lab and reduce drinking under natural conditions. However, the results did not show significant differences in craving and alcohol consumption between the fenofibrate and placebo groups. Reverse translational studies in rodent models confirmed the lack of fenofibrate effect at human-equivalent doses. These findings suggest that inadequate translation of drug dose from rodents to humans may account for the lack of fenofibrate effects on alcohol craving and consumption in humans with AUD. The results highlight the need for new brain-penetrant PPAR-α agonists to adequately test the therapeutic potential of PPAR-α agonists for AUD, and the importance of reverse translational approaches and selection of human-equivalent doses in drug development.

1. Introduction

1.1. Background

Alcohol Use Disorder (AUD) is a chronic relapsing disorder characterized by repeating cycles of pathological alcohol use and acute withdrawal (American Psychiatric Association, DSM-5 Task Force, 2013), followed by protracted abstinence symptoms of negative affect and craving (Koob and Mason, 2016). Worldwide, alcohol misuse is the seventh leading risk factor for premature death and disability (GBD 2016 Alcohol Collaborators, 2018), and AUD affects approximately 11.3% of Americans (Substance Abuse and Mental Health Services Administration, 2021). Alcohol misuse is the primary reason given for 1,714,757 emergency department visits (Agency for Healthcare Research and Quality, 2016) and more than 140,000 deaths annually in the United States (Centers for Disease Control and Prevention, 2020; (National Institute on Alcohol Abuse and Alcoholism, 2023), with cost estimates of $249 billion annually primarily for medical care and lost productivity (U.S. Department of Health and Human Services, 2016).

Currently, there are three drugs approved by the US Food and Drug Administration (FDA) for the treatment of AUD: oral disulfiram, oral and injectable naltrexone, and oral acamprosate. Despite their availability, these drugs are underutilized. Given the diverse biological processes that contribute to AUD, there is an urgent need to provide a broader spectrum of pharmacotherapies to advance the AUD treatment field (Jonas et al., 2014; Mark et al., 2009; Kranzler and Soyka, 2018).

Stress has been implicated in the initiation of, maintenance of, and relapse to alcohol misuse, as well as in the development of AUD (Koob, 2008; Sinha, 2008). Chronic stress can lead to neuroinflammation, which has been associated with increased vulnerability to alcohol use

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and AUD (Crews et al., 2017). Neuroinflammation is characterized by the activation of microglia and astrocytes, the release of pro-inflammatory cytokines, and the production of reactive oxygen species (ROS), which can contribute to alcohol-induced neuro-degeneration and cognitive deficits (Crews et al., 2017). Neuro-inflammation can also drive the brain stress systems (Breese et al., 2008; Knapp et al., 2011; Koob et al., 2021), thus stress can drive neuro-inflammation and neuroinflammation can drive stress, and both can be exacerbated by alcohol misuse. As a result, targeting neuroinflammation and its underlying mechanisms may provide a novel approach to treating AUD and mitigating the effects of stress on alcohol use.

Recent advances in the neurobiology of AUD have identified numerous potential drug targets in the domain of negative affect and neuroinflammation (Koob and Mason, 2016). One promising avenue for exploration is the role of peroxisome proliferator receptors (PPARs) in AUD treatment. PPARs are part of the nuclear hormone receptor superfamily and can act as ligand-activated transcription factors (Mangelsdorf et al., 1995), with three subtypes (alpha, beta, and gamma) that each have distinct physiological functions (Heneka and Landreth, 2007). Historically prescribed for the treatment of cardiovascular disease and diabetes (Rosenson et al., 2012; Chigurupati et al., 2015), PPAR agonists have also been associated with anti-inflammatory effects, which may be relevant to AUD treatment given the link between pro-inflammatory processes and vulnerability to drinking relapse (Crews et al., 2017).

Preclinical studies have shown promising results for PPAR-α and -γ agonists in animal models of AUD (Matheson and Le Foll, 2020; Ferguson et al., 2014; Stopponi et al., 2011, 2013). However, human studies have been limited, and some PPAR-γ agonists, such as pioglitazone, have shown increased craving in response to alcohol stimuli and heightened risk of myopathy, leading to the termination of a human laboratory study (Schwandt et al., 2020). Despite these setbacks, the PPAR-α agonist fenofibrate has demonstrated potential in reducing alcohol consumption in mice and rats (Karahanian et al., 2014; Ferguson et al., 2014; Blednov et al., 2015; Haile and Kosten, 2017; Rivera-Meza et al., 2017), with a strong neuronal signature in mouse brain and targeting of GABA interneurons in the amygdala (Ferguson et al., 2014). These findings are particularly relevant given the known adaptations in GABA interneurons in the central nucleus of the amygdala in alcohol dependence that may drive excessive alcohol seeking (Roberto et al., 2008). Moreover, a recent study (Ibáñez et al., 2023) demonstrated that fenofibrate administration during the withdrawal stage after chronic ethanol consumption in rats reduced voluntary alcohol intake by 80% during post-abstinence relapse and decreased ethanol-induced neuro-inflammation and oxidative stress in the brain, further supporting the potential of fenofibrate in AUD treatment. Furthermore, a human genome-wide association study has shown an association between a single nucleotide polymorphism in PPAR-α and alcohol withdrawal (Blednov et al., 2015), suggesting that transcriptional regulators can be targeted with small molecule pharmaceuticals (Savarese and Lasek, 2018). These findings provide a compelling rationale for testing fenofibrate in a human laboratory model of risk factors for drinking relapse in AUD. Fenofibrate (TRICOR, Abbott Laboratories) is available as tablets for oral administration and is FDA-approved for treatment of hypercholesterolemia and hypertriglyceridemia, at a maximum dose of 145 mg per day.

1.2. Specific objectives

The aim of this human laboratory study was to translate the results of preclinical behavioral and genomic studies suggesting PPAR-α agonist efficacy for AUD to a non-treatment seeking human population with current AUD. We hypothesized that, relative to placebo, fenofibrate 145 mg/d would significantly attenuate responsiveness to *in vivo* alcohol cues in the lab, and reduce drinking under natural conditions, and thus provide a rational basis for later-phase testing of fenofibrate as a potential therapeutic for AUD.

2. Material and methods

2.1. Human laboratory study

2.1.1. Trial design

We used a validated human laboratory model of risk factors for drinking relapse in early abstinence (Mason et al., 2009; Vendruscolo et al., 2015) to generate the first human data on short-term efficacy and safety of the highest FDA-approved dose of oral fenofibrate (145 mg/d, no titration) relative to double-blind placebo in 50 non-treatment-seeking male and female paid volunteers with AUD. Subjects were randomly assigned in a simple 1:1 ratio to double-blind treatment with fenofibrate (n = 25) or matched placebo (n = 25) in a parallel groups design. Dosing duration was 9 days based on time to achieve fenofibrate steady state plasma concentration. Subjects were permitted ad libitum drinking while on drug, except for required abstinence on the last 3 days of the 9-day dosing period, in order to test the effect of fenofibrate on responsivity to alcohol cues in the lab when motivational signs of early abstinence are manifest.

2.1.2. Participants

Participants were males and females, 18–65 years of age, who met Diagnostic and Statistical Manual for Mental Disorders – Fifth Edition (DSM-5; American Psychiatric Association, DSM-5 Task Force, 2013 criteria for Alcohol Use Disorder (AUD) ≥ moderate severity, and who were not seeking treatment and willing to take daily oral medication for research purposes. Subjects were not pregnant, did not have a urine drug screen positive for substances of abuse other than alcohol, were not taking disallowed medications and did not have significant medical or psychiatric disorders that would increase potential risk or interfere with study outcomes, as detemined by the study physician's review of medical history, vital signs, routine urine and blood tests, electrocardiogram, and physical examination. The Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar; Sullivan et al., 1989) was used to assess severity of alcohol withdrawal symptoms. Subjects were required to have a negative breathalyzer reading, a negative urine dipstick for alcohol glucuronide, and a CIWA-Ar score <9 at randomization and prior to testing, to eliminate acute alcohol or withdrawal effects on study measures. IRB-approved print, social media and internet advertisements were used to recruit participants. Participants were paid $50 for completing the screening, $75 for completing the randomization visit, $150 for completing the laboratory session, and $100 for completing a follow-up visit.

2.1.3. Setting

This single-site study was conducted in the outpatient clinical research unit of the Laboratory of Clinical Neuropsychopharmacology, Pearson Center for Alcohol and Addiction Research, Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037.

2.1.4. Intervention

Medication conditions. Fenofibrate was purchased and over encapsulated with gelatin #0 capsules to match placebo by University Compounding Pharmacy in San Diego, CA. Double-blind study drug was given without titration in gelatin #0 capsules, administered orally as one 145 mg capsule of fenofibrate or identical placebo in the AM, taken with or without food. Double-blind study drug was packaged in a blistercard with the subject's study ID number and the day and time of each dose indicated on the blistercard. Study drugs, packaging and dosing regimen were identical to preserve the double-blind. Medication adherence was verified with returned blistercard and pill count. Plasma was obtained on the last day of dosing, frozen (−80F) and analyzed in batch after study completion for determination of fenofibrate concentration in the laboratory of Thomas Cooper, Nathan Kline Institute, Orangebuurg, NY to verify correct medication assignment per the randomization code and ingestion of active medication. Results were

examined for an association with outcome on an exploratory basis, as fenofibrate has no established therapeutic plasma level.

Cue Reactivity Procedures. Affective (positive, negative, neutral) and *in vivo* beverage (alcohol or water) cue reactivity manipulations, followed by 4 Visual Analogue Scale craving ratings (VAS; adapted from the Alcohol Craving Questionnaire [Singleton et al., 1995]), were conducted in the laboratory at the conclusion of the Days 1–9 dosing/Days 7–9 abstinent interval. The subject was seated in an upholstered arm chair in a sound and light-attenuated room and their preferred alcoholic beverage or bottled water were presented on a tray in random order for 90 s following each mood condition (positive, negative, and neutral pictures selected from the International Affective Picture System [Lang et al., 2008]). The subject was told to view and sniff the beverage for 90 s but not to drink it, and then complete the four Visual Analogue Scale (VAS) craving items after each of the six affect-beverage pairings. Subjects received all affect X beverage cue combinations in random order.

2.1.5. Outcome measures

Efficacy. Craving in response to alcohol cues in abstinent individuals with AUD has been shown to be predictive of subsequent drinking relapse (Vendruscolo et al., 2015), and thus VAS craving ratings in response to *in vivo* alcohol cue exposure in the laboratory comprised the primary outcome for this proof-of-concept study, with higher scores indicating greater craving (range 0–20). The four VAS items were: 1. Strength: How strong is your craving to drink alcohol? 2.Impulse: If I could drink alcohol now, I would drink it. 3. Control: It would be hard to turn down a drink right now. 4. Relief: Having a drink would make things just perfect. The Timeline Followback Interview (TLFB; Sobell and Sobell, 1992) was the secondary measure used to assess daily intake of standard drinks consumed over the 6-day period of ad libitum drinking on study drug. A standard drink contains ~14 g of pure alcohol such that a 12 ounce can of beer is eqivalent to 5 ounces of table wine or 1.5 ounces of distilled spirits. Self-reported drinking data were collected as a secondary measure, as the 3-day abstinent interval constrained complete evaluation of this variable, and drinking data were collected under less controlled conditions than craving in response to *in vivo* beverage cue exposure in the laboratory. A daily drinking diary was used to verify TLFB data.

Safety. Adverse drug experiences were recorded on standardized case report forms that depicted each side effect complaint in terms of its onset, duration, severity, relation to study medication and clinical action. Vital signs, routine urine and blood tests, EKG and physical exam were conducted pre and post treatment to verify that no clinically significant changes from baseline had occurred.

2.1.6. Sample size

There were no previous clinical studies of fenofibrate for AUD to use in power calculations. Accordingly, we used acamprosate as the reference compound for calculating sample size because it is the most recent drug approved for AUD and novel drugs would be expected to exceed its effect size to merit further development. Data from our acamprosate proof-of-concept study using cue reactivity with total VAS craving ratings as the outcome identified a moderate effect size for acamprosate (66.1%). This effect size is based on a total VAS craving score of 5.0 ± 1.40 in subjects tested on acamprosate (n = 20) and 16.7 ± 3.83 in subjects tested on placebo (n = 20), with a coefficient of variation of 100% for each group. Based on these data, and assuming a similar or better effect size for drinking would be found, 20 completed subjects per treatment group provides adequate power (80%) to detect an effect size of 66.1% at a two-sided significance level of 0.05. Therefore, randomizing 50 subjects (25 per treatment group) allowed for a generous estimate (based on completed studies) of 2–3 noncompliant subjects and 2–3 dropouts for a total of 20 completed subjects per arm.

2.1.7. Randomization and blinding

The study statistician generated the simple 1:1 randomization sequence using the open-access program found at http://randomization.com. The pharmacy technician alone had access to the doubleblind code throughout the study. Subjects and all personnel with subject contact (the medical assistant, the study coordinator, the study physicians and investigators) were blind to treatment until the study was completed. Randomization key and study medication were kept in a locked cabinet not accessible to study staff, and electronic data were maintained in a dedicated, password-protected database.

2.2. Methods for rat models

2.2.1. Animals

Adult male Wistar rats (n = 12; Charles River, Raleigh, NC, USA), weighing 225–275 g at the beginning of the experiments, were housed in pairs per cage in a temperature-controlled (22 °C) vivarium under a 12 h/12 h light/dark cycle (lights on at 8:00 p.m.), with ad libitum access to food and water. All behavioral tests were conducted during the dark phase of the light/dark cycle. All procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.2.2. Operant alcohol self-administration

Self-administration sessions were conducted in standard operant conditioning chambers (Med Associates, St. Albans, VT, USA). The animals were first trained to self-administer 10% alcohol (v/v) and water solutions until stable responding was maintained. To facilitate the acquisition of operant self-administration, the rats were initially provided with free-choice access to 10% alcohol (v/v) and water for one day in their home cages to habituate them to the taste of alcohol. Subsequently, the rats were subjected to an overnight session in the operant chambers with access to one lever (right lever) that delivered water on a fixed-ratio 1 (FR1) schedule of reinforcement. Food was available ad libitum during this training. After one day off, the rats were subjected to a 2-h session on an FR1 schedule for one day, followed by a 1-h session on an FR1 schedule the next day, with one lever delivering alcohol (right lever). All subsequent sessions lasted 30 min, with two levers available (left lever: water; right lever: alcohol) until stable levels of intake were reached.

2.2.3. Alcohol vapor chambers

The rats were made dependent by chronic intermittent exposure to alcohol vapors as previously described (Gilpin et al., 2008; O'Dell et al., 2004). They underwent cycles of 14 h ON (BALs during vapor exposure ranged between 150 and 250 mg%) and 10 h OFF, during which behavioral testing for acute withdrawal occurred (i.e., 6–8 h after the vapor was turned OFF when brain and blood alcohol levels are negligible (Gilpin et al., 2009). In this model, rats exhibit somatic withdrawal signs and negative emotional symptoms, reflected by anxiety-like responses and elevated brain reward thresholds (de Guglielmo et al., 2016; Edwards et al., 2012; O'Dell et al., 2004; Rimondini et al., 2002; Schulteis et al., 1995).

2.2.4. Operant self-administration during alcohol vapor exposure

Behavioral testing during alcohol vapor exposure occurred three times per week. The rats were tested for alcohol (and water) self-administration on an FR1 schedule in 30-min sessions during acute withdrawal from alcohol (i.e., 6–8 h after vapor was turned off when brain and blood alcohol levels are negligible). Operant self-administration on an FR1 schedule requires minimal effort by the animal to obtain the reinforcement and herein was considered a measure of intake.

2.3. Methods for mice models

2.3.1. Animals

C57BL/6J male mice (n = 84, Jackson Labs East) were single-housed for the entire duration of the study. The mice were maintained on a 12-h/12-h light/dark cycle with ad libitum access to food and water with 7090 Teklad sani-chips (Envigo) as bedding for the home cages and experimentation. All behavioral tests were conducted during the dark phase of the light/dark cycle. All procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.3.2. Drinking in the dark (DID)

A standard binge drinking protocol, DID, was used for the alcohol-drinking group (Thiele and Navarro, 2014). In this voluntary drinking protocol, the mice received 3 days of 2 h/day access to a single bottle containing either water or 20% alcohol in their home cage, starting 3 h after lights off. On the 4th day, the mice were given 4 h of access to a bottle containing 20% alcohol. A water bottle was available in the cage for all animals during non-testing periods. At the end of the 4-h drinking period, the mice were bled (tail) for determination of blood alcohol levels (BALs). In the first experiment, 36 male C57BL/6J mice (Jackson Labs East) underwent the 4-day Drinking-in-the-Dark (DID) protocol as previously described (Thiele and Navarro, 2014; Rhodes et al., 2005). The protocol was repeated twice with a one-week interval between tests.

2.3.3. Two-bottle choice/CIE vapor (mice)

We used the 2BCE/CIE paradigm, a well-established mouse model of alcohol dependence, to induce alcohol dependence, the escalation of alcohol drinking, and behavioral symptoms of abstinence. In the 2BCE/CIE paradigm, weeks of voluntary alcohol drinking during limited-access 2BCE sessions are alternated with weeks of CIE.During 2BCE weeks, the mice were given access to two bottles containing water and alcohol (15%, vol/vol), respectively, Monday through Friday for 2 h, starting at the beginning of the dark phase of the circadian cycle. During CIE weeks, the mice were exposed to four cycles of 16-h intoxication/8-h abstinence (Monday through Friday) followed by 72-h abstinence (Friday to Monday). Each 16-h period of alcohol vapor exposure was primed with an intraperitoneal injection of alcohol (1.5 g/kg) to initiate intoxication and pyrazole (an alcohol dehydrogenase inhibitor, 1 mmol/kg) to normalize alcohol clearance rates between individual mice. Average blood alcohol levels were measured periodically at the end of alcohol vapor inhalation periods and averaged *175*–250 mg%. Air-exposed mice received injections of pyrazole only.

The mice were first given 2 weeks of 2BCE (weeks 1–2) and were then split into two groups with equivalent baseline intake (nondependent [2BCE/Air], n = 24; alcohol-dependent [2BCE/CIE], n = 24). The mice were then subjected to four rounds of alternating weeks of Air/CIE exposure with weeks of 2BCE drinking.

2.4. Statistical analysis

2.4.1. Data analysis

All data were analyzed using GraphPad Prism software (version 9.0, GraphPad Software, San Diego, CA, USA) for animal studies and R software for human laboratory cue reactivity data.

2.4.2. Analysis for human laboratory cue reactivity data

The human laboratory cue reactivity data were analyzed using a repeated measures, within-subjects design specifying a mixed-effects, random-intercept linear model with strength of craving as the outcome variable. Within-subject predictors included affect (negative, positive, neutral) and beverage (alcohol, water) cue conditions, with drug condition as a between-subject predictor. The model was estimated with REML in R using the package lme4 v.1.1-x (Bates et al., 2015). The

accepted level of significance for all of the tests was P < 0.05 (2-tail).

2.4.3. Analysis for rat models

For the rat study, a paired *t*-test was used to compare reinforced responses for alcohol before and after exposure to the vapor chamber. A two-way repeated measures ANOVA was conducted to assess the effects of fenofibrate treatment on alcohol self-administration and water consumption, with treatment (fenofibrate dose) as the between-subjects factor and time as the within-subjects factor. Post hoc analyses were performed using Tukey's multiple comparisons test when a significant interaction was observed.

2.4.4. Analysis for mouse models

For the DID test, a two-way repeated measures ANOVA was conducted to assess the effects of fenofibrate treatment on ethanol consumption, with treatment (fenofibrate dose) as the between-subjects factor and time as the within-subjects factor. Post hoc analyses were performed using Tukey's multiple comparisons test when a significant interaction was observed. For the 2BCE/CIE paradigm, a two-sample *t*-test was used to compare ethanol drinking between CIE experimental and control mice at baseline. Due to the large group difference, the effects of fenofibrate treatment on ethanol consumption were analyzed separately for control and experimental groups using a two-way repeated measures ANOVA, with treatment (fenofibrate dose) as the between-subjects factor and time as the within-subjects factor. Post hoc analyses were performed using Tukey's multiple comparisons test when a significant interaction was observed.

In all cases, the level of significance was set at p < 0.05.

3. Results

3.1. Human laboratory study

3.1.1. Baseline characteristics, participant flow and medication adherence

Subjects were 39 males and 11 females with mean age = 37.6 years (SD = 12.0) who met DSM-5 criteria for severe AUD (DSM-5 mean symptom count = 6.3, SD =1.7); ≥6 symptoms is considered "severe" AUD (Table 1). The study was completed by 22 of 25 (88%) subjects in each treatment group (Fig. 1). Rate of medication adherence was 97% by returned pill count. Determination of fenofibrate concentration in human plasma showed detectable levels of fenofibrate in all subjects randomized to fenofibrate, and no detectable levels of fenofibrate in subjects randomized to placebo.

3.1.2. Efficacy

Results showed significantly greater craving in response to alcohol cues relative to water cues (β = 4.23, SE(β) = 0.97, t(42) = 4.23, p = 0.000013) but no differences in craving between fenofibrate and placebo were observed (β = −1.86, SE(β) = 1.97, t(42) = −0.94, p = 0.51) (see Fig. 2 for individual VAS items). Similarly, no pre-post (Day 0 – Day 9) differences were found in measures of alcohol consumption between treatment groups (β = −0.91, SE(β) = 1.37, t(42) = -0.66, p = 0.34) (Table 2). Fenofibrate concentration in plasma did not correlate significantly with alcohol cue reactivity or drinking measures. Fenofibrate effects also did not vary as a function of sex or other baseline demographic and clinical characteristics summarized in Table 1.

3.1.3. Safety

Fenofibrate was safe and well-tolerated in this AUD sample. There were no serious or unexpected adverse events (AEs). No AE was reported by more than two subjects and all AEs were reported as mild or moderate in severity. One fenofibrate-treated subject discontinued treatment due to complaints of nausea, dizziness and weakness that subsequently resolved.

Table 1

Baseline demographic and clinical characteristics of fenofibrate and placebo groups (n = 50)[a].

	Placebo N = 25	Fenofibrate N = 25	P-value
Sex (self-identified)			
Male	18 (72%)	21 (84%)	0.50
Female	7 (28%)	4 (16%)	
Race (self-identified)			
American Indian/Alaska Native	0 (0%)	3 (12%)	0.42
Asian	7 (28%)	4 (16%)	
Black/African American	3 (12%)	1 (4%)	
Native Hawaiian/other Pacific Islander	0 (0%)	1 (4%)	
White	12 (48%)	8 (32%)	
>1 Race	2 (8%)	2 (8%)	
Unknown/Decline	1 (4%)	1 (4%)	
Ethnicity (self-identified)			
Hispanic/Latino	2 (8%)	6 (24%)	0.25
Not Hispanic/Latino	23 (92%)	19 (76%)	
Age, years	36.6 (11.8)	38.5 (12.4)	0.59
DSM-5 AUD symptoms, number[b]	6.4 (1.9)	8.0 (1.6)	0.89
Alcohol use history			
Age of 1st drink, years	14.8 (2.7)	14.4 (2.0)	0.51
Age of onset of alcohol-related problems, years[c]	20.4 (3.3)	20.0 (5.1)	0.78
Years of heavy drinking[d]	13.4 (10.2)	18.2 (11.6)	0.13
Alcohol use, 90 days pre-treatment			
Drinks per day, number[e]	7.7 (3.6)	7.8 (3.2)	0.87
% Drinking days	85.7 (16.3)	82.3 (17.98)	0.49
% Heavy drinking days[d]	73.7 (24.1)	74.5 (23.2)	0.90

[a] All values are means (standard deviations) unless otherwise specified.

[b] Numbers >5 indicate severe AUD. Drug N = 24.

[c] Placebo N = 21; Drug N = 23.

[d] Heavy drinking is defined as ≥5 drinks per day (males), ≥4 drinks per day (females).

[e] A standard drink contains the equivalent of 14 grams of pure alcohol, e.g., 12oz. beer, 5oz. wine or 1.5oz. distilled spirits.

3.2. Rat study

In the rat study, the results demonstrated a significant increase in reinforced responses for alcohol after exposure to the vapor chamber. Before the vapor chamber exposure, rats exhibited an average of 21 reinforced responses for alcohol. This number increased to 45 reinforced responses after the vapor chamber exposure, indicating a substantial escalation in alcohol self-administration ($t_{11} = 4.973$, $p < 0.001$, Fig. 3). At this point, treatment commenced, and animals were orally injected in a counterbalanced order with fenofibrate (0.0, 10, 50, and 100 mg/kg) 1 h before the test. Doses were selected based on the FDA Guideline for Human to Rat conversion of doses. Considering a ~600 g rat (0.07 m2 of body surface area) and a 60 kg human (1.6 m2 of body surface area), the dose tested in humans in this work (145 mg) would correspond to 9.6 mg/kg. Therefore, we tested 10, 50, and 100 mg/kg to account for the higher metabolic rates and possibly lower bioavailability in rats. Results demonstrated that fenofibrate treatment, even at doses ten times higher than the human equivalent dose, was unable to reduce alcohol self-administration in ethanol-dependent rats ($F_{3,33} = 1.86$; $p > 0.05$, Fig. 3, panel A). Furthermore, the treatment had no significant effects on water consumption ($F_{3,33} = 0.30$; $p > 0.05$, Fig. 3, panel B).

3.3. Mouse studies

3.3.1 Drinking in the Dark (DID): For the first DID test, mice were treated acutely with 0, 50, or 100 mg/kg fenofibrate p.o., 1 h before bottle exposure only on the 4th day. For the second DID test, mice were treated with 0, 50, or 100 mg/kg fenofibrate each of the 4 days, 1 h before the drinking sessions. Ethanol consumption was measured at two time points: 2 h and 4 h after bottle exposure (Fig. 4A). There was a significant Treatment × Time interaction observed following a single administration of fenofibrate in test 1 ($F2,32 = 4.167$; $p < 0.05$). However, the Tukey's post-hoc analysis did not reveal any significant differences at any doses during this test (Fig. 4A). This suggests that while there was an interaction between treatment and time, the specific doses of fenofibrate administered in this test did not lead to significant differences in the measured outcomes. In the second test, the 100 mg/kg fenofibrate dose showed a trend towards decreased ethanol drinking on days 2 and 3 of administration, and on day 4, this dose appeared to reduce ethanol consumption across the 4-h period. However, the two-way ANOVA did not reveal any significant interaction between time and treatment ($F_{2, 32} = 1.293$, $p > 0.05$). This indicates that while there was a noticeable trend in the reduction of alcohol consumption with the 100 mg/kg dose, the statistical analysis did not support a significant effect of chronic fenofibrate treatment on ethanol consumption in this test (Fig. 4A).

3.3.1. Two-bottle choice/CIE vapor

In the second experiment, 48 male C57BL/6J mice underwent the standard CIE-2BC protocol as described before (Becker and Lopez, 2004). One vapor mouse was lost due to illness. Observing the baseline day, there was a significant difference in ethanol drinking between CIE experimental and control mice ($t_{45} = 4.19$; $p < 0.001$, Fig. 4B), confirming that the procedure induced escalation of drinking. Due to this large group difference, dose effects were investigated separately in the control and experimental groups. Fenofibrate did not significantly decrease ethanol drinking in the control mice at either dose on any of the four days ($F_{8, 84} = 1.709$; $p > 0.05$, Fig. 4C). In the alcohol dependent mice, while the treatment with 100 mg/kg fenofibrate seemed to show a trend of decreased ethanol consumption on day 4, the Anova analysis did not show any Time × Treatment interaction ($F_{8, 80} = 1.007$; $p > 0.05$, Fig. 4C).

4. Discussion

In attempting to reconcile our negative clinical findings with positive pre-clinical data reported in the literature, we considered 5 potential explanations: the human laboratory model did not elicit sufficient craving to show drug versus placebo effects, medication non-adherence, treatment misassignment, inadequate treatment duration and inadequate dosing. Significantly greater craving elicited in response to alcohol than water cues supports the validity of the model, and the robustness of craving elicited by *in vivo* alcohol cues was sufficient to detect drug-placebo differences in prior studies assessing other drug effects in the same model (Vendruscolo et al., 2015; Mason et al., 2009). The congruence between drinking outcomes and craving elicited in the lab across both negative and positive studies supports the predictive validity of the lab model for detecting treatment effects on drinking outcomes. Fenofibrate plasma levels consistent with the randomization code ruled out medication non-adherence or treatment misassignment. Effects of PPAR's on ethanol consumption in rodent models of AUD can be immediate and typically do not change with repeat dosing, making inadequate treatment duration in humans an unlikely explanation. We studied the highest FDA-approved dose of fenofibrate (145 mg), and were constrained from testing higher doses due to risk of increases in serum transaminases [AST (SGOT) or ALT (SGPT)], which is also a risk of heavy alcohol use.

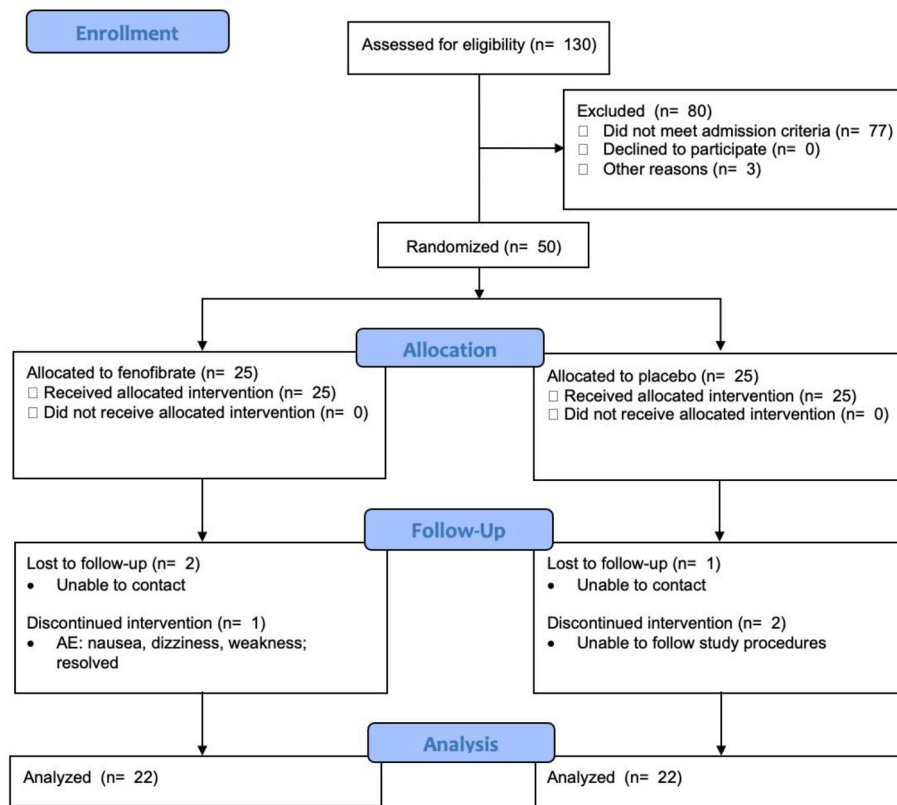Prior research using mouse models of AUD showed a dose dependent

Fig. 1. Fenofibrate CONSORT 2010 flow diagram.

effect of fenofibrate, with 150 mg/kg showing optimal effects in decreasing ethanol seeking and consumption in both male and female mice in an intermittent (every other day) paradigm (Blednov et al., 2015). This dose in mice is equivalent to a 740 mg dose in a 60kg/132.3lb human, which exceeds the highest FDA-approved dose by > 5-fold. Fenofibrate has poor brain penetrance and a likely explanation for the negative human findings is that the available human dose was far below that needed to sufficiently activate the PPAR-α signaling needed to drive the effects of fenofibrate on ethanol seeking and consumption found in animal models.

We used a reverse translational approach that tested the human equivalent dose in mouse and rat models of AUD to replicate the lack of an ethanol effect found in humans treated with fenofibrate 145 mg/d. Our negative pre-clinical findings lend support to our hypothesis that the highest FDA-approved dose of fenofibrate in humans was inadequate to sufficiently activate the PPAR-α signaling needed to drive the effects of fenofibrate on ethanol seeking and consumption found in animal models.

These reverse translational studies confirm a lack of fenofibrate effect at doses equivalent to the 145 mg human dose, across mouse and rat species, in models of AUD commensurate with prior studies showing efficacy at doses >5x the available human dose. These results support the explanation that inadequate translation of a drug dose from rodents to humans accounted for a lack of fenofibrate effects on alcohol craving and consumption in humans with AUD. These results do not necessarily refute the therapeutic potential of PPAR-α agonists for AUD, but rather underscore the need for new brain-penetrant PPAR-α agonists that permit adequate testing of the human equivalent dose of the 150 mg/kg dose consistently associated with fenofibrate efficacy in mouse models

of AUD. Importantly, the 150 mg/kg dose was not associated with sex-specific effects found at lower doses in mouse models of AUD, and sometimes associated with fenofibrate when studied for other indications. Recently, chemically modified variants of fenofibrate have been developed to increase its stability, water solubility, and tissue penetration to optimize fenofibrate's anticancer potential (Stalinska et al., 2019). Such chemical variations of fenofibrate that increase brain penetrability may ultimately be exploited for treating AUD.

5. Conclusions

In conclusion, our study highlights the importance of considering the translation of drug doses from animal models to human trials in the development of new treatments for AUD. While fenofibrate showed promise in animal models, the FDA-approved dose for humans was insufficient to produce the desired effects on alcohol craving and consumption. This calls for further research into the development of new brain-penetrant PPAR-α agonists that can be tested at doses consistent with those shown to be effective in animal models, as well as the exploration of chemically modified variants of fenofibrate that may have increased brain penetrability. Importantly, the development of new brain-penetrant PPAR-α agonists or chemically modified variants of fenofibrate may not only target alcohol craving and consumption but also address stress-induced neuroinflammation. By modulating the neuroimmune response and reducing the detrimental effects of stress on the brain, these novel compounds could provide a comprehensive approach to treating AUD, particularly in individuals with a history of chronic stress exposure.
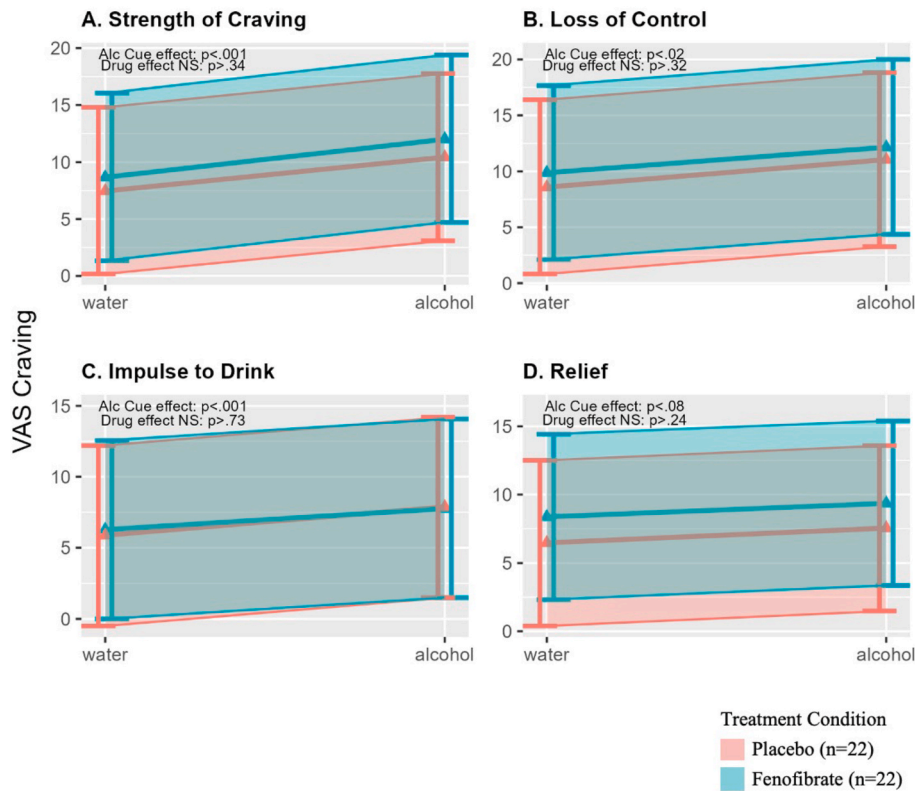
Fig. 2. Effects of fenofibrate (145 mg/d) vs. placebo on alcohol cue reactivity. Alcohol cues elicited significantly stronger craving than water cues across 4 visual analogue scale (VAS) measures of craving. Responses did not vary as a function of treatment. Data are presented as means and 95% confidence intervals.

Table 2
Effects of fenofibrate versus placebo on alcohol use (Days 1–9).

	Placebo N = 23	Fenofibrate N = 23	P-value
Drinks per day[a]	3.8 (1.7)	4.7 (2.5)	0.16
% Drinking days	58.0 (13.0)	59.0 (16.7)	0.81
% Heavy drinking days	38.1 (21.8)	42.0 (22.7)	0.55
Change: drinks per day (post-pre)[a]	−3.6 (3.6)	−2.7 (1.8)	0.34
Change: % drinking days (post-pre)	−26.5 (18.0)	−23.4 (14.9)	0.53
Change: % heavy drinking days (post-pre)[b]	−33.4 (28.0)	−32.1 (22.5)	0.85

[a] A standard drink contains the equivalent of 14 g of pure alcohol, e.g., 12oz. beer, 5oz. wine or 1.5oz. distilled spirits.
[b] Heavy drinking is defined as ≥5 drinks per day (males), ≥4 drinks per day (females).

6. Registration and approvals

The human laboratory study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Scripps Research Institutional Review Board (protocol #14–6362), was conducted under an Investigator-initiated IND (#122524), and was registered on ClinicalTrials.gov (NCT02158273). All subjects provided written informed consent prior to study participation.

The preclinical procedures adhered to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.
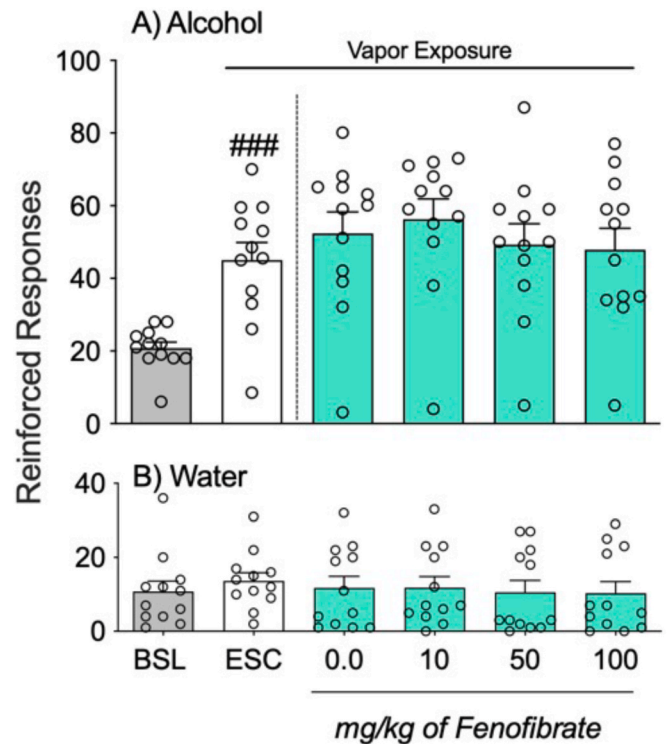

Fig. 3. Effects of Fenofibrate on dependence-induced escalation of alcohol drinking in Wistar rats. Effect of fenofibrate on **A)** alcohol intake and **B)** water intake. ###p < 0.01 Escalation (ECS) vs Baseline (BSL).
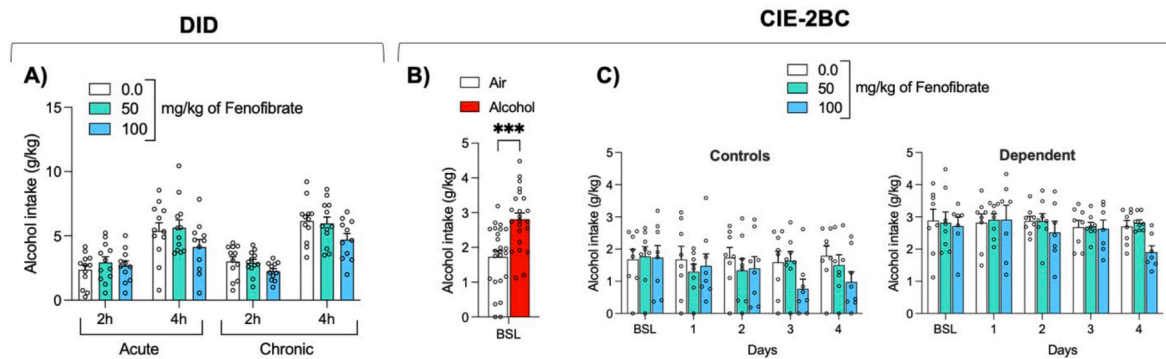
DID / CIE-2BC

Fig. 4. Effects of Fenofibrate on DID (acute and chronic administration) and on CIE-2BC in C57BL/6J mice. **A)** Ethanol intake on the 4th day of the DID test in mice treated acutely (left) or across all 4 days (right) with fenofibrate, measured at 2 h (2h) and 4 h (4h) after bottle exposure. **B)** Escalation of drinking in the 2-bottle-choice model (2BCE) after chronic intermittent exposure (CIE) to alcohol vapor. **C)** Ethanol intake (baseline and 4 days of administration) in mice treated with fenofibrate. Non-dependent control mice are shown in the left panel and CIE dependent experimental mice are shown in the right panel. ***$p < 0.01$ Alcohol CIE vs Air controls.

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Data sharing

Data will be made available upon request.

CRediT authorship contribution statement

Barbara J. Mason: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **David Estey:** Investigation, Writing – review & editing. **Amanda Roberts:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Giordano de Guglielmo:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Olivier George:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **John Light:** Data curation, Formal analysis, Visualization, Writing – review & editing. **Mike Stoolmiller:** Data curation, Formal analysis, Writing – review & editing. **Susan Quello:** Data curation, Validation, Writing – review & editing. **Michael Skinner:** Supervision, Writing – review & editing. **Farhad Shadan:** Investigation, Writing – review & editing. **Adnan Begovic:** Investigation, Writing – review & editing. **Mark C. Kyle:** Investigation, Writing – review & editing. **R. Adron Harris:** Conceptualization, Writing – review & editing.

Declaration of competing interest

BJM has served as a consultant for Awakn Life Sciences Corp. and Imrium Therapeutics. All other authors report no biomedical financial interests or potential conflicts of interest.

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