A Pilot Study of the Safety and Initial Efficacy of Ivermectin for the Treatment of Alcohol Use Disorder

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Background: Ivermectin (IVM) is an antiparasitic agent that has been shown to reduce alcohol intake in mice, suggesting IVM as a potential treatment for alcohol use disorder (AUD). However, the safety profile of IVM administered in combination with an intoxicating dose of alcohol has not been characterized in humans.

Methods: This pilot project sought to provide the first clinical evidence that IVM could be repositioned as an AUD pharmacotherapy by examining (i) the safety of combining IVM (30 mg oral , once a day [QD]) with an intoxicating dose of intravenous alcohol (0.08 g/dl) and (ii) the effects of IVM on alcohol cue-induced craving and subjective response to alcohol. Eleven individuals with AUD participated in a randomized, placebo-controlled, crossover study in which they received the study medication, participated in a cue exposure paradigm followed by intravenous alcohol administration, and remained in an inpatient unit overnight for observation.

Results: IVM treatment, versus placebo, did not increase the number or severity of adverse effects during alcohol administration or throughout the visit. However, IVM did not reduce cue-induced craving nor did it significantly affect subjective response to alcohol.

Conclusions: These results suggest that IVM (30 mg oral, QD) is safe in combination with an intoxicating dose of alcohol, but do not provide evidence that this dose of IVM is effective in reducing alcohol craving or its reinforcing effects. Given the preclinical data suggesting IVM is effective in reducing alcohol consumption in mice, additional studies testing larger samples and alternate dosing regimens are warranted to further characterize the potential efficacy of IVM as an AUD treatment.

Key Words: Alcohol Use Disorder, Pharmacotherapy, Medication Development, Safety, Pharma cokinetcs, Pilot Laboratory Study.

OF THE 18 million people in the United States who suffer from alcohol use disorder (AUD), only an estimated 13% receive specialized treatment for their addiction (Litten et al., 2012; Miller et al., 2011). One factor that undoubtedly contributes to this low treatment rate is the limited number of medications approved by the Food and Drug Administration (FDA) to treat AUD. Further compounding this issue, these few available medications have only modest treatment efficacy (Rösner et al., 2010a,b). Recent reviews have outlined the National Institute on Alcohol Abuse and Alcoholism's (NIAAA's) vision to improve available pharmacological treatment options to treat AUD (Litten et al., 2012). One such strategy emphasized by the NIAAA is to identify and validate novel molecular targets that can be used to develop additional pharmacotherapies for AUD (Litten et al., 2012).

One novel target, the P2X receptor (P2XR), is a family of cation-permeable ligand-gated ion channels activated by synaptically released extracellular adenosine 5′-triphosphate. This receptor family has garnered significant attention as a possible target for AUD pharmacotherapies (for review, see Franklin et al., 2014). Preclinical studies have shown that alcohol acts as a negative allosteric modulator for P2XRs, as low alcohol concentrations (~5 mM) can produce rapid inhibition of P2XR function (Asatryan et al., 2008; Franklin et al., 2014; Kidd et al., 1995; Li et al., 1993). Furthermore, *p2rx4* gene expression in the brain of rats is negatively associated with alcohol consumption and preference (Kimpel et al., 2007; Tabakoff et al., 2009). These findings suggest that alcohol alters the function of P2X4Rs, and the P2X4R is involved in alcohol consumption in rodents.

Ivermectin (IVM), a semi synthetic macrocyclic lactone, is an FDA-approved broad-spectrum antiparasitic avermectin (Geary, 2005). While IVM's antiparasitic effects are attributed to action on a nonmammalian, glutamate-gated inhibitory chloride channel (Cully et al., 1994; Dent et al., 1997), IVM is also a selective positive allosteric modulator of P2X4Rs (Jelinkova et al., 2006; Silberberg et al., 2007)

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and acts on P2X4R sites that are thought to be modulated by alcohol (Asatryan et al., 2008; Popova et al., 2010). Recent evidence suggests that IVM blocks the inhibitory effect of alcohol in vitro (Asatryan et al., 2010) and is able to reduce alcohol intake and preference in mice due in part to its action on P2X4Rs (Wyatt et al., 2014; Yardley et al., 2012). The doses of IVM needed to produce these anti-alcohol effects in mice appear to be well tolerated, safe, and show no evidence of abuse liability (Bortolato et al., 2013). Thus, IVM appears to be a promising, novel therapeutic for AUD.

Despite the promising results in rodents, the efficacy of IVM for the treatment of AUD has not been examined in humans. Additionally, few studies to date have investigated the effects of ethanol (EtOH) on IVM safety and pharmacokinetics (PK). Retrospective self-reports of IVM and alcohol co-use have not found an increased association with serious adverse events (Takougang et al., 2008). Yet, a subintoxicating dose of EtOH (Shu et al., 2000) and IVM preparations in an alcoholic solution (Edwards et al., 1988) both increased IVM's bioavailability, suggesting that alcohol could potentially influence IVM efficacy and adverse effects. Further complicating matters, preclinical studies have indicated that the dose of IVM required for the treatment of AUD may be at least twice as high as the 200 μg/kg FDA-approved IVM dose (Yardley et al., 2012). Therefore, even though IVM has been shown to be safe and tolerable at 10 times the FDA-recommended dosing (Guzzo et al., 2002), the safety and PK effects of combining a higher-than-approved IVM dose with controlled alcohol administration still needs to be demonstrated before IVM can be developed as an AUD treatment.

Human laboratory models have been useful in medication development for AUD by measuring markers of safety and tolerability and elucidating the biobehavioral mechanisms by which pharmacotherapies may be efficacious (Plebani et al., 2012; Ray et al., 2010a,b). Therefore, the primary goal of this randomized, double blind, placebo-controlled, crossover, human laboratory pilot study was to determine the safety and tolerability of administering IVM (30 mg oral, once a day [QD]) in combination with an intoxicating dose of alcohol (0.08 g/dl). A secondary study goal was to examine the initial efficacy of IVM in reducing alcohol cue-induced craving and affecting subjective response to alcohol in a sample of nontreatment seeking individuals with AUD. IVM PK were also assessed to ensure that study measures were administered at times corresponding to peak medication bioavailability. This pilot safety study represents the first step in the clinical development of IVM as a treatment for AUD.

MATERIALS AND METHODS

The study protocol and all procedures were approved by the Institutional Review Board of the University of California, Los Angeles (UCLA) and conducted in accordance with the Declaration of Helsinki.

Participants and Screening Procedures

A community sample of nontreatment seeking drinkers was recruited via online and print advertisements in the Los Angeles area. Interested individuals called the laboratory to complete a preliminary telephone-screening interview used to assess general eligibility requirements. Inclusion criteria included the following: (i) aged between 21 and 65 years; (ii) met DSM-V criteria for current AUD; (iii) consumed 48 or more drinks per month; and (iv) fluent in English. Exclusion criteria consisted of the following: (i) were currently in a treatment program for alcohol problems, had been in treatment for alcohol use in the 30 days before study enrollment, or were seeking treatment for alcohol use; (ii) reported clinically significant alcohol withdrawal symptoms on the Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised; (iii) self-reported use of any nonprescription drugs (excluding marijuana) or met DSM-V criteria for a nonalcohol substance use disorder; (iv) self-reported diagnosis of or met DSM-V criteria for any psychiatric disorders; (v) being pregnant, as verified by a urine pregnancy test; (vi) having a body mass index (BMI) < 18.5 or >30; or (vii) reporting any medical conditions or medications that would be contraindicated with taking IVM.

Eligible individuals were invited to the laboratory for an in-person screening visit after a 24-hour abstinence period, where they received a full explanation of study procedures and provided written informed consent. After consenting, participants were required to blow into a breathalyzer to demonstrate a breath alcohol concentration (BrAC) of 0.000 g/dl, and urine toxicology and pregnancy tests were performed. Participants who tested positive for alcohol, drug use, or pregnancy were excluded from participation. Participants then completed a number of baseline questionnaires and interviews, outlined in the "Measures" section below. Participants received $40 for participating in the screening visit.

Participants deemed eligible following the in-person screening visit were invited to complete a physical examination with the study physician, consisting of medical history, a clinical laboratory panel, and BMI calculation, to ensure medical eligibility for the study. Individuals who passed the physical examination were invited to participate in the experimental procedures, detailed below. Participants received $20 for participating in the medical screening visit. A total of 74 participants (20% female) completed the in-person screening visit, 27 of whom were eligible. Seventeen participants were screened by the physician for medical eligibility, 3 of whom were ineligible and 3 of whom decided not to continue with the experimental procedures. Eleven individuals ($n = 2$ female) were randomized to a medication sequence, and all 11 participants completed both experimental sessions.

Experimental Procedures

Eligible participants completed 2 experimental sessions in a randomized, counterbalanced, and crossover fashion at the UCLA Clinical and Translational Research Center (CTRC). Participants were asked to abstain from drinking alcohol for 24 hours prior to scheduled sessions; they were also asked to fast the morning of the session. After participants arrived at the laboratory at 8:00 AM, abstinence from alcohol and recreational drugs (excluding marijuana) was immediately verified via breathalyzer and urine toxicology screen, respectively. Women also provided a negative urine pregnancy test at this time. At 9:00 AM, participants received a single 30-mg dose of IVM (or matched placebo), administered by CTRC nursing staff. Participants ate calorie-controlled, standardized meals at 8:00 AM (breakfast), 11:30 AM (lunch), and 2:30 PM (snack) totaling 70% of daily estimated kilocalories. At regular intervals throughout the day, participants reported subjective adverse effects and alcohol craving and provided blood samples for

IVM PK analysis. In the afternoon, participants completed cue reactivity and alcohol infusion paradigms. Participants who were smokers were allowed smoke breaks as needed until the start of the cue reactivity procedure to avoid potential nicotine withdrawal effects on mood.

At 3:00 PM (i.e., 6 hours after medication administration), participants completed a cue reactivity paradigm, following well-established guidelines (Monti et al., 1987, 2001). During this task, participants were asked to listen to a 5-minute guided cue exposure script, during which they were exposed to both a neutral beverage (a glass of water) and their preferred alcoholic beverage in a fixed order to avoid carryover effects. Prior to beginning the paradigm and after each cue exposure, participants completed a questionnaire to assess alcohol craving.

Following the cue reactivity paradigm, at 3:45 PM, participants completed an alcohol infusion session (O'Connor et al., 1998; Ray et al., 2012, 2013). Participants were seated in a reclining chair, and a 6% EtOH solution was administered intravenously in their nondominant arm. Alcohol was administered intravenously to effectively control blood alcohol levels, at the following rates, taking into account participant's sex and weight: for males, 0.166 ml/min × weight (kg); for females, 0.126 ml/min × weight (kg). Target BrACs were 0.020, 0.040, 0.060, and 0.080 g/dl. At each target BrAC, the infusion rate was reduced to half to maintain a stable BrAC level, and participants then completed a battery of subjective measures. Heart rate and blood pressure were also recorded at each target BrAC. After completion of the alcohol infusion and removal of the intravenous line, participants also completed subjective measures during the descending limb of intoxication at BrAC levels of 0.060 and 0.040 g/dl.

Participants were provided a meal following the alcohol infusion procedures and were monitored by CTRC nursing staff overnight. After overnight observation, participants were discharged the following morning (Day 2), returned again the next day (Day 3) for a final blood draw and to complete follow-up questionnaires, and were contacted by phone 7 days after the experimental session to assess potential adverse effects after Day 3. At their second Day 3 visit, participants received an individual session of motivational interviewing, delivered by a licensed clinical psychologist or a clinical psychology PhD student under the supervision of a licensed clinician. Experimental sessions were scheduled at least 7 days apart to avoid any carryover effects (17.8 ± 10.9 days, mean ± SD). Participants received $340 for completing all experimental procedures.

Measures

Screening Measures. Participants completed a battery of assessments during the screening process that assessed basic demographics (e.g., age, education), drinking and drug use behavior (e.g., Alcohol Use Disorders Identification Test; Reinert and Allen, 2002), and depressive symptoms (e.g., Beck Depression InventoryII [BDI-II]; Beck et al., 1996). The following interviews were also performed: (i) 30-day Timeline Follow-Back (Sobell and Sobell, 1992), used to obtain estimates of daily alcohol, cigarette, and marijuana use; (ii) Structured Clinical Interview for DSM-V (First et al., 1995), used to assess for AUD and other exclusion criteria, such as nonalcohol substance use or other current psychiatric disorders; and (iii) Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised (Sullivan et al., 1989), a 10-item scale to assess for alcohol withdrawal. All clinical interviews were conducted by masters' level clinicians under the PI's supervision.

Arrival and Daily Measures. On the day of each experimental session, upon arrival to the laboratory and before medication administration, baseline depressive symptoms (BDI-II), anxiety symptoms (Beck Anxiety Inventory; Beck and Steer, 1990), alcohol craving (Penn Alcohol Craving Scale; Flannery et al., 1999), and overall mood (Profile of Mood States [POMS]; Curran et al., 1995) were assessed. After medication administration (9:00 AM), alcohol craving and adverse effects were regularly assessed until the start of the cue reactivity paradigm (3:00 PM, 6 hours postmedication). Craving was repeatedly assessed at 0, 2, 4, and 6 hours after medication administration using the Alcohol Urge Questionnaire (AUQ; Bohn et al., 1995), which consisted of 8 items associated with urge to drink alcohol, rated on a 7-point scale (1 = Strongly Disagree, 7 = Strongly Agree). Potential medication-related adverse effects were repeatedly assessed using the Systematic Assessment for Treatment Emergent Events (SAFTEE; Jacobson et al., 1986) at 0, 1, 2, 4, and 6 hours after medication administration. The SAFTEE is a 24-item checklist in which the participant can identify whether a symptom is present (yes/no), its severity (mild, moderate, severe), and whether it was caused by the medication (yes/no). Participants also completed the AUQ and SAFTEE 24 and 48 hours (Days 2 and 3, respectively) after medication administration and were administered the SAFTEE over the phone 7 days after the experimental session day to monitor whether the participants had experienced any adverse effects after their Day 3 visit.

Cue Reactivity and Alcohol Infusion. Participants completed the AUQ during the cue reactivity paradigm (baseline, postwater cue, and postalcohol cue). The following measures were administered during the alcohol infusion sessions (0.000, 0.020, 0.040, 0.060, and 0.080 g/dl): (i) the AUQ; (ii) the Biphasic Alcohol Effects Scale (BAES; Martin et al., 1993), a 14-item scale designed to capture the stimulant and sedative effects of alcohol, each rated on an 11-point scale (0 = Not at all, 10 = Extremely); (iii) the Drug Effects Questionnaire (DEQ; Johanson and Uhlenhuth, 1980), a 4-item questionnaire that captures subjective effects using the questions "Do you feel any drug effects?," "Do you like the effects you are feeling right now?," "Would you like more of the drug right now?," and "Are you high?" each rated on an 11-point scale; (iv) an 8-item version of the POMS, consisting of 8 adjectives rated on a 5-point scale (0 = Not at all, 4 = Extremely) designed to capture 4 dimensions of mood: tension (consisting of uneasy and anxious), vigor (lively, energetic), negative affect (downhearted, discouraged), and positive affect (cheerful, joyful); and (v) the Subjective High Assessment Scale (SHAS; Schuckit, 1984), consisting of 13-items on a 10-point Likert scale ranging from "not at all" to "extremely" used to assess subjective feelings of alcohol intoxication. The SAFTEE was administered at BrAC levels of 0.00, 0.040, and 0.080 g/dl.

PK Sampling Procedures and Measures. Blood samples (4 ml) were collected from participants' nondominant arm at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, and 48 hours post-IVM administration. Plasma samples were analyzed as previously described, where the lowest level of quantification was 5 ng/ml with a range of 1 to 1,000 ng/ml and an accuracy of 90 to 110% (Yardley et al., 2012). PK parameters were calculated using a noncompartmental analysis: (i) Maximum plasma concentration (C_{max}; ng/ml) for each subject, (ii) area under the curve (AUC) from 0 to 48 hours after IVM administration, (iii) time to C_{max} (T_{max}, time), and (iv) half-life ($T½$ hours).

IVM Dose Selection and Timing of Measures. A single IVM dose of 30 mg was chosen to ensure an optimal balance of participant safety and drug efficacy. Acute administration of a single dose of IVM, ranging from 2.5 to 10 mg/kg, reduced alcohol consumption and preference in mice, with maximum efficacy observed between 5 and 10 mg/kg (Yardley et al., 2012). Also, the lowest IVM dose that was detectable in the brains of mice and reduced alcohol consumption was 2.5 mg/kg (Yardley et al., 2012). In humans, IVM is FDA approved for a single dose of 200 μg/kg (e.g., ~13.6 mg for a 150 lb person) but has been shown to be safe up to 120 mg (Guzzo et al., 2002). However, no human laboratory studies have coadministered an intoxicating dose of alcohol and IVM.

Therefore, for this initial pilot study, we selected the lowest dose of IVM that was both effective in reducing alcohol consumption in murine studies (i.e., 3.1 mg/kg; Yardley et al., 2012) and also shown to be safe and tolerable in humans (Guzzo et al., 2002). Using allometric scaling (Anderson and Holford, 2009), the human equivalent dose to 3.1 mg/kg in mice was determined to be 30 mg. The UCLA Research Pharmacy provided IVM and encapsulated the medication into one 30-mg capsule.

The peak effects of IVM in reducing alcohol intake in mice corresponded to the C_{max} and T_{max}, which occurred ~8 hours after IVM administration (Yardley et al., 2012). As IVM PK are comparable between mice and humans (Guzzo et al., 2002; Yardley et al., 2012), the timing of the alcohol cue reactivity paradigm was chosen to begin at the time when IVM is likely to be producing central effects (~6 hours after IVM administration) and the entirety of the alcohol infusion procedures was chosen to correspond with projected peak central effects of 30 mg IVM (i.e., ~7 to 9 hours after IVM administration).

Statistical Analysis

Repeated measures analyses of variance (ANOVAs) were used to analyze the effects of IVM on cue-induced craving and subjective response to alcohol infusion. Repeated measures ANOVAs were also used to compare arrival mood and craving measures, BrAC levels during infusion, and all repeated daily mood, adverse effect, and craving measures. All repeated measures ANOVAs included dose (IVM or placebo) as a within-subject factor. Time was additionally included as a within-subject factor, with varying levels, for the following analyses: (i) *Postmedication/precue exposure measures*: SAFTEE, 5 levels; AUQ, 4 levels; (ii) *Cue exposure*: AUQ 3 levels; (iii) *Alcohol infusion*: SAFTEE, 3 levels; AUQ, SHAS, DEQ, and BAES, 6 levels; (iv) *Postalcohol infusion, descending limb*: AUQ, SHAS, DEQ, and BAES, 2 levels. The SAFTEE was analyzed as (i) number of adverse effects reported per time point (potential range: 0 to 24) and (ii) the severity of each reported adverse effect per time point (potential range: 1 to 3). The severity of an adverse effect was only analyzed if an adverse effect was reported at that time point, resulting in an unequal N for the severity measure across all time points for each subject. Because of this, adverse event severity was analyzed separately using individual t-tests for each time points. Covariates were considered (e.g., sex, age) but ultimately rejected because in the crossover design participants serve as their own controls. An alpha threshold of 0.05 was set for all statistical analyses. Significant effects were explored with simple effects post hoc testing. All analyses were performed with SPSS 22.0 (Released 2013, IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY). Power and effect size calculations were performed with SPSS and G*Power version 3.1.9.2 (Faul et al., 2009). Based on the sample of 11 participants, the current study had 80% power to detect an effect size between 0.2 and 0.4 (Cohen's f, medium effect size) for subjective measures assessed during the cue reactivity and alcohol infusion paradigms (within participant correlations between 0.63 and 0.92). Cohen's f is presented in the results section as an index of effect size (f values of 0.10, 0.25, and 0.40 represent small, medium, and large effect sizes, respectively) for outcomes related to the alcohol cue reactivity and alcohol infusion paradigms.

RESULTS

Sample Characteristics and Arrival Measures

Sample characteristics for the 11 participants are reported in Table 1. There were no significant differences between sessions in mood or craving upon arrival to the laboratory (Table 2).

Table 1. Sample Characteristics

Variable	Mean (SD) or %	Range
Age	38.82 (11.39)	24 to 58
Sex, % Male	82	–
Ethnicity,% Caucasian	46	–
Education (years)	14.18 (2.27)	10 to 16
BMI (kg/m^2)	23.90 (2.58)	20.78 to 29.01
BDI-II	5.45 (4.61)	0 to 14
AUDIT	20.18 (6.16)	9 to 29
CIWA-AR	1.55 (2.07)	0 to 6
DSM-V current AUD severity	2.00 (0.90)	1 to 3
Number of drinking days[a]	18.91 (6.25)	8 to 27
Drinks per drinking day[a]	8.90 (3.67)	3.47 to 14.78
Heavy drinking days[a]	13.36 (3.67)	4 to 24
Cigarette smokers, %	55	–
Cigarettes per day[a]	8.62 (6.16)	2 to 20
Marijuana smokers, %	45	–
Number of days smoking marijuana[a]	17.40 (10.60)	2 to 30

AUD, alcohol use disorder; AUDIT, Alcohol Use Disorders Identification Test; BMI, body mass index; BDI-II, Beck Depression Inventory-II; CIWA-AR, Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised.
[a]Over past 30 days, as determined by Timeline Follow-Back interview. Data obtained at behavioral screen.

Table 2. Arrival Measures and Infusion BrAC Levels

	Ivermectin	Placebo
Arrival measures		
BAI	1.64 (2.73)	0.45 (0.82)
BDI-II	3.00 (4.22)	3.00 (4.15)
PACS	13.82 (6.54)	12.45 (6.64)
POMS		
Tension	0.73 (0.43)	0.79 (0.79)
Vigor	1.64 (0.69)	1.36 (0.91)
Negative affect	0.55 (0.42)	0.57 (0.32)
Positive affect	2.54 (1.28)	2.04 (0.92)
Target BrAC (g/dl)		
Infusion		
0.02	0.022 (0.002)	0.022 (0.003)
0.04	0.041 (0.002)	0.041 (0.013)
0.06	0.063 (0.002)	0.062 (0.002)
0.08	0.083 (0.002)	0.081 (0.001)
Postinfusion		
0.06	0.057 (0.003)	0.059 (0.003)
0.04	0.040 (0.002)	0.040 (0.003)
Time to reach target BrAC from previous time point (minutes)		
Infusion		
0.02	13 (2)	12 (2)
0.04	18 (4)	18 (4)
0.06	26 (4)	25 (5)
0.08	29 (9)	28 (5)
Postinfusion		
0.06	21 (9)	22 (6)
0.04	55 (21)	58 (27)

BAI, Beck Anxiety Inventory; BDI-II, Beck Depression Inventory-II; BrAC, breath alcohol concentration; PACS, Penn Alcohol Craving Scale; POMS, Profile of Mood States, 40 item version.
Data are mean (SD). POMS was assessed at 9:00 AM, all other arrival measures were collected at 8:00 AM.

PK Analysis

Average IVM concentrations across the study time points are reported in Fig. 1. Mean C_{max} was 406.03 ± 398.36 ng/

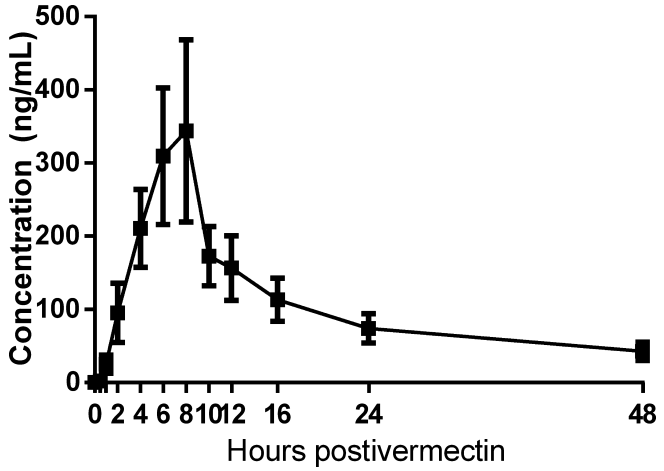

Fig. 1. Ivermectin (IVM) pharmacokinetics (PK). Mean (±SEM) IVM concentrations at baseline (0), 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, and 48 hours post-IVM administration. Additional PK measures (means ± SD) are as follows: C_{max} = 406.03 ± 398.36 ng/ml; AUC = 5,078 ± 4,258; T_{max} = 9.09 ± 3.62 hours; $T½$ = 15.75 ± 6.86 hours. AUC, area under the curve.

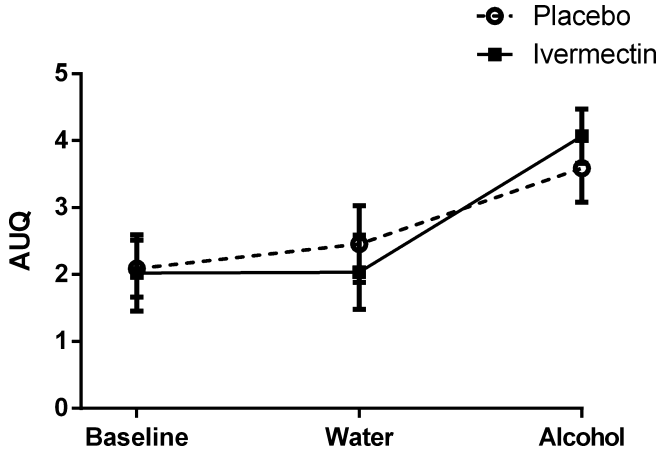

Fig. 2. Alcohol cue reactivity. The alcohol cue significantly increased Alcohol Urge Questionnaire (AUQ) craving compared to baseline and the water cue. However, ivermectin (IVM), versus placebo, did not affect AUQ craving during the cue reactivity paradigm. Data are presented as mean (±SEM).

ml (mean ± SD) leading to a mean IVM exposure over 0 to 48 hours ($AUC_{0–48}$) 5,078 ± 4,258 (ng*h/ml). Peak concentration (T_{max}) was found 9.09 ± 3.62 hours after IVM administration, where mean $T½$ was 15.75 ± 6.86 hours.

Cue Reactivity and Alcohol Infusion

The alcohol cue significantly increased AUQ craving compared to baseline and the water cue, Time main effect: F (2, 9) = 16.7, $p < 0.001$, Cohen's $f = 1.30$; Post hoc: Alcohol cue > Baseline and Water cue, $p < 0.01$. IVM, versus placebo, however, did not affect this response ($p = 0.99$, Cohen's $f = 0.00$; Fig. 2).

There were no significant differences between target infusion and postinfusion BrAC levels or time to reach BrAC targets between sessions (Table 2). Table 3 lists the number of individual adverse effects experienced during alcohol infusion as measured by the SAFTEE. During alcohol infusion, the average number of reported adverse effects ($p = 0.71$, Cohen's $f = 0.12$) and severity of each reported adverse effect ($ps > 0.44$, Cohen's $ds > 0.01$ and <0.47) did not differ between IVM and placebo sessions (Fig. 3). Alcohol infusion increased DEQ "like," "feel," "high," and "more," and SHAS intoxication and decreased POMS tension (Time main effects, $ps < 0.05$, Cohen's $fs > 0.66$ and <1.70; Fig. 4), but did not affect AUQ craving, BAES stimulation or sedation, or any other POMS subscale ($ps > 0.09$, Cohen's $fs > 0.25$ and <0.47). However, IVM, versus placebo, did not affect subjective response on any measure during alcohol infusion or during the postinfusion, declining BrAC time points ($ps > 0.19$, Cohen's $fs > 0.05$ and <0.20). None of the sample characteristics in Table 1 were significant covariates of the responses to IVM.

Table 3. Number of Individual Systematic Assessment for Treatment Emergent Event Adverse Effects Reported During Alcohol Infusion

	Placebo			Ivermectin		
Adverse effect	0.00	0.04	0.08	0.00	0.04	0.08
1. Abdominal pain or cramps	0	1	1	0	0	0
2. Yellow eyes	0	0	0	0	0	0
3. Nausea or vomiting	0	0	0	0	0	0
4. Irritability or anger	1	0	0	0	0	0
5. Increased desire for sex	1	0	1	1	2	1
6. Nervousness	1	0	0	1	0	0
7. Ringing in the ears	0	0	0	0	0	0
8. Decrease in appetite	0	0	0	0	0	0
9. Depression	0	0	0	0	0	0
10. Fatigue	1	2	2	1	1	2
11. Difficulty in staying awake	3	3	4	3	2	2
12. Increase in appetite	0	1	3	1	2	4
13. Blurred vision	1	1	1	0	1	1
14. Drowsiness	2	4	2	2	4	5
15. Headache	0	0	0	0	1	0
16. Night sweats	0	0	0	0	0	0
17. Mental confusion	0	0	0	0	0	0
18. Anxiety	0	0	0	0	1	0
19. Joint or muscle pain	0	0	0	0	0	0
20. Dizziness	0	0	0	0	1	2
21. Sexual problems	0	0	0	0	0	0
22. Difficulty sleeping	0	0	0	0	0	0
23. Fever or chills	0	0	0	0	0	0
24. Decreased desire for sex	0	0	0	0	0	0

Daily Measures

For the first 6-hour postmedication administration, IVM, versus placebo, did not affect AUQ craving, nor the average number or severity of reported adverse effects. Similarly, IVM, versus placebo, did not affect AUQ craving or either SAFTEE outcome 24- and 48-hour postmedication administration. Finally, the average number and severity of reported adverse effects collected 7 days after the experimental session did not differ between IVM and placebo sessions.

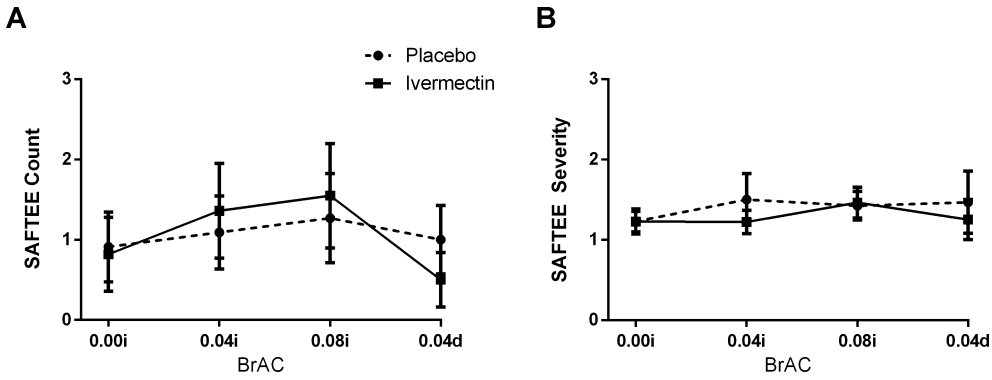

Fig. 3. Adverse effects during alcohol infusion. The average number (A) and severity (B) of reported adverse effects on the Systematic Assessment for Treatment Emergent Event (SAFTEE) were not affected by alcohol and did not differ between ivermectin (IVM) and placebo sessions. On the *x*-axis, 0.00i, 0.04i, and 0.08i refer to BrAC during alcohol infusion, while 0.04d refers to breath alcohol concentration (BrAC) postalcohol infusion. Data are presented as mean (±SEM).

DISCUSSION

The current pilot laboratory study was the first to examine the safety and initial efficacy of coadministration of a single 30-mg dose of IVM and intravenous alcohol infusion. This dose of IVM was safe and well tolerated both on its own and during the alcohol infusion. The number and severity of reported adverse effects were low and did not differ from the placebo session. Conversely, IVM showed no efficacy at this dose in reducing alcohol cue-induced craving or basal alcohol craving throughout the day, nor did it affect subjective response to alcohol infusion. These findings are an important first step in developing IVM as a treatment for AUD and may inform future studies testing IVM or other members of the avermectin family as potential treatments for AUD.

The primary goal of this phase 1 pilot study, to establish the safety of IVM (30 mg) independently and in combination with alcohol, was successful. Confirming the safety of a novel medication is a required, and often difficult, first step in the clinical development of any drug (Litten et al., 2012). A fundamental challenge in translational medication development is transitioning a novel medication from preclinical research to successfully testing the medication in a phase 1 trial (Litten et al., 2012). The gap between preclinical and phase 1 testing, often referred to as the "Valley of Death," has obstructed progression in the development of numerous promising novel medications (Litten et al., 2012). Therefore, establishing the safety and tolerability combining IVM at a dosage above the FDA-approved quantity with an intoxicating dose of alcohol is a necessary landmark in developing IVM for treating AUD.

IVM did not reduce basal or cue-induced craving and failed to affect subjective response to alcohol, all of which are considered potential markers of medication efficacy (Ray et al., 2010b). There are several potential factors that may have contributed to these null results, but it seems particularly likely that a higher IVM dose may be needed to produce anti-alcohol effects in humans. As the maximum IVM concentration (C_{max}: 406.03 ± 398.36 ng/ml) occurred at a time (T_{max}: 9.09 ± 3.62 hours) when subjective response to alcohol was being measured, we would have expected to detect a medication effect if this IVM dose was effective. Taken together, these results suggest that the 30-mg IVM dose administered in the present study, while higher than the FDA-approved dose, may not have been high enough to affect response to alcohol and alcohol craving. In rodents, single, acute IVM doses, ranging from 2.5 to 10 mg/kg, reduced alcohol consumption and preference, with maximum efficacy observed between 5 and 10 mg/kg (Yardley et al., 2012). The absence of studies in the literature reporting the safety of coadministering an intoxicating dose of alcohol and IVM led us to select the lowest IVM dose that produced anti-alcohol effects in mice and was also safe in humans (Guzzo et al., 2002). However, given the safety profile of IVM that was demonstrated in the present study and by others (Guzzo et al., 2002), future studies should consider examining a higher dose of IVM that is comparable to the optimal range of 5 to 10 mg/kg observed in mice. An equivalent dose within this range may increase the likelihood of detecting anti-alcohol use effects without producing safety and tolerability concerns.

An additional factor that might have contributed to null efficacy findings may relate to the choice of measures administered in the current study. Forced alcohol administration and alcohol cue reactivity are reliable methods used to measure subjective response to alcohol and alcohol craving, respectively (Ray et al., 2016). However, several promising pharmacotherapies for AUD have not reduced the positive subjective effects of alcohol in the laboratory (reviewed elsewhere, e.g., Ray et al., 2016). Thus, IVM could feasibly have utility in treating AUD without affecting subjective response to alcohol. For example, it may instead act by reducing motivation to consume alcohol or alleviating protracted withdrawal symptoms. The current design would not capture such mechanisms of action. Given that IVM reduces alcohol

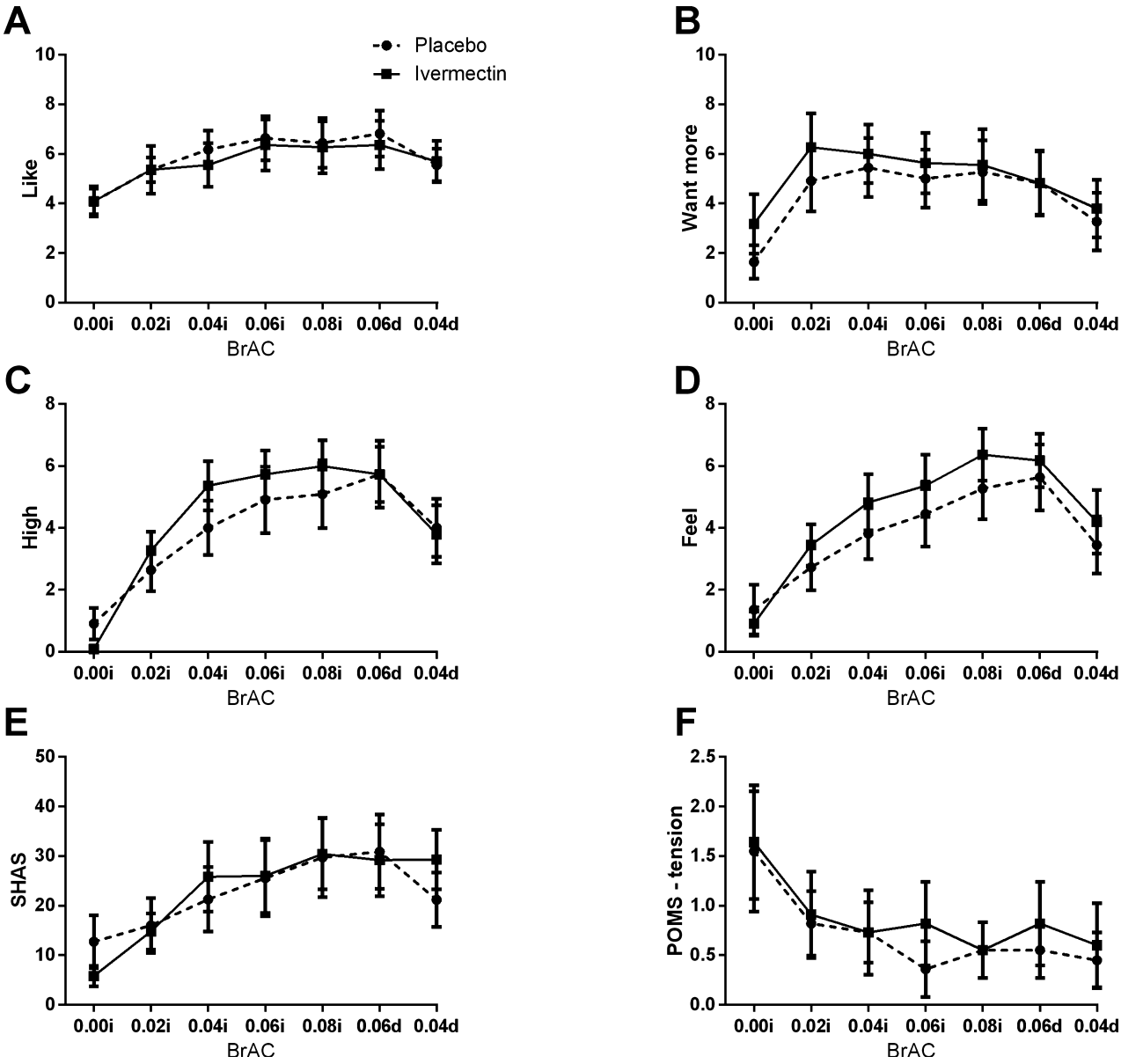

Fig. 4. Subjective response to alcohol. Alcohol infusion increased Drug Effects Questionnaire (DEQ) "like (A)," "more (B)," "high (C)," and "feel (D)," and Subjective High Assessment Scale (SHAS) intoxication (E) and decreased Profile of Mood States (POMS) tension (F). On the *x*-axis, 0.00i, 0.02i, 0.04i, and 0.08i refer to breath alcohol concentration (BrAC) during alcohol infusion, whereas 0.06d and 0.04d refer to BrAC postalcohol infusion. Data are presented as mean (±SEM).

self-administration in rodents (Yardley et al., 2012) and is also a positive allosteric modulator at GABA-A receptors (Bortolato et al., 2013), both aforementioned behavioral markers may be sensitive to the effects of IVM. To capture the full translational potential of IVM, future studies should consider employing both a higher dose of IVM and including additional paradigms, such as an alcohol self-administration session, in their design.

A comparison of the IVM PK parameters that were obtained in this study with other human and rodent IVM studies may also aid in the interpretation of the present findings. Our IVM PK results in humans were generally similar to those reported in a mouse study that found IVM was effective in reducing alcohol consumption (Yardley et al., 2012); however, the total IVM exposure (as measured by AUC) in the current study was comparatively lower, which may in part explain the discrepant medication effects between the 2 studies (Yardley et al., 2012). In comparison with human study that administered a single 30-mg IVM dose (Guzzo et al., 2002), the C_{max} (~56% greater) and T_{max} (~97% greater) reported in the present study were considerably greater than previously reported values. One possibility for the noticeable discrepancy between these values is that prior studies have found alcohol increases plasma concentration of IVM (Edwards et al., 1988; Shu et al., 2000). Although the increase in IVM bioavailability did not corre-

spond to increases in adverse effects or medication efficacy, future alcohol studies administering IVM doses >30 mg should consider this altered PK profile.

The results of the present study should be examined in light of its strengths and limitations. The strengths of the study include its highly translational nature, its within subject and crossover design, and its use of well-established human laboratory paradigms with putative clinical significance as outcome measures (e.g., alcohol cue exposure and alcohol infusion). Additionally, all experimental procedures being conducted in a highly controlled inpatient environment allowed the regular assessment of adverse effects for 24 continuous hours. As with all pilot studies, the primary study limitation is a small sample size that potentially limited our ability to detect significance. While the crossover design may have mitigated some loss in statistical power, the high variability in pharmacotherapy response that is typically observed in such studies may have eliminated these gains. The alcohol infusion did not significantly change ratings of BAES stimulation or sedation, AUQ craving, and POMS negative mood, positive mood, or vigor despite producing medium effect sizes on these outcomes (Cohen's *f*s > 0.25 and <0.47), which may be indicative of a lack of statistical power. Importantly, this lack of alcohol effect could have contributed to null IVM effects on many of these measures (i.e., if there is no alcohol effect, there is no effect for IVM to augment or diminish). Yet, alcohol did produce sizeable increases on the SHAS and DEQ want, feel, like, and high items, as well as a decrease on POMS tension (Cohen's *f*s > 0.66 and <1.70, large effect sizes), providing several measures that could have captured potential IVM effects. Furthermore, IVM produced only small, nonsignificant effects on subjective response to alcohol or to alcohol-related cues (Cohen's *f*s > 0.00 and <0.20), and therefore, we are confident that the null IVM findings reported in the article were primarily due to a lack of medication effect rather than a lack of alcohol effect or low statistical power.

In summary, the current pilot study found that IVM (30 mg oral, QD) was safe in combination with an intoxicating dose of alcohol but did not display efficacy in reducing alcohol craving or affecting subjective response to alcohol. We advise against interpreting the null initial efficacy results of this pilot study as an indication that IVM is not a promising AUD pharmacotherapy or that the P2XR family is not a promising target for treating this disorder. Indeed, other members from IVM's class of drugs, such as abamectin, have also been shown to decrease alcohol intake and preference in mice through actions at the P2X4R (Asatryan et al., 2014). The study and its results as a whole are promising for several reasons: It is an excellent example of a translational study, it provides support for the safety of IVM, and it identified methodological changes that future studies should employ when testing this medication for AUD (e.g., higher dosage, additional measures). These strengths speak to the importance of using human laboratory studies to effectively translate preclinical findings and fostering working collaborations between preclinical and clinical scientists to facilitate the development of novel treatments for AUD. Given the paucity and limited efficacy of available pharmacotherapies, as well as IVM's strong preclinical findings and its safety and tolerability, IVM and other avermectins warrant investigation as potential AUD pharmacotherapies.

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CONFLICTS OF INTEREST

Lara Ray is a paid consultant for GSK and has received medication from Pfizer and Medicinova. Daryl Davies is an inventor on a patent for the use of IVM for the treatment of AUD.

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