Effects of Pioglitazone on Stress Reactivity and Alcohol Craving

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ABSTRACT

Alcohol use disorder (AUD) is associated with significant negative health, social, and economic costs. Alcohol activates the hypothalamic-pituitary-adrenocortical (HPA)-axis, and stress can increase the reward value of drinking. Continued excessive drinking results in behavioral changes marked by increased compulsive drinking, stress reactivity, and neuro-inflammation[8]. FDA-approved pharmacotherapies for AUD (e.g., naltrexone, acamprosate, disulfiram) are effective in less than a third of treated individuals[9]. *Importantly, none of the currently approved medications for AUD directly address stress reactivity, underscoring the need to develop novel pharmacotherapies targeting stress-related processes associated with AUD.*

A promising pharmacological target for addressing AUD and stress reactivity is the family of peroxisome proliferator-activated receptors (PPARs), proteins that act as ligand-activated transcription factors that exhibit both anti-inflammatory and neuro-protective responses in the brain[10]. A growing number of pre-clinical models have shown promising effects of PPAR agonists on drug-related outcomes[11]. For example, the PPARγ agonist pioglitazone *1) significantly reduced free-access alcohol consumption; and 2) attenuated alcohol consumption and withdrawal symptoms following stress exposure in rats bred to highly prefer alcohol*[12,13]. An important innovation of the current study will be to translate these results for the first time to humans with AUD.

The primary purpose of the current proposal is to obtain preliminary data to increase the likelihood of funding for a future NIH grant, while balancing the feasibility of completing the current proposal. To that end, we will utilize a within-subject, repeated measures design to assess the feasibility of administering pioglitazone for four weeks to non-treatment and treatment-seeking individuals with AUD and elevated levels of stress/anxiety **(N=20)**. Participants will undergo pre- and post-treatment human laboratory assessment of stress-reactivity and stress-induced alcohol craving. During treatment, changes in alcohol use, stress/anxiety, and alcohol craving will be assessed in the natural environment. The study design features 1) rigorous monitoring of medication compliance (Medication Event Monitoring System (MEMs)) and alcohol use (urinary ethyl glucuronide (ETG)); 2) biological assessment of stress reactivity (salivary cortisol, blood pressure, and heart rate); and 3) a multi-dimensional assessment of stress-induced alcohol craving incorporating both traditional measures of alcohol craving in addition to relatively novel behavioral economic measures (delay discounting, alcohol demand). Taking these measures together, we hope to present a robust and comprehensive characterization of pioglitazone on alcohol use, stress reactivity, and stress-induced alcohol craving.

SPECIFIC AIMS

Specific Aim 1: To assess pre- to post-treatment reductions in stress reactivity (self-report, salivary cortisol, heart rate, blood pressure) and stress-induced alcohol craving (self-report, alcohol demand) in the human laboratory setting.

Hypothesis 1: *We will observe pre- to post- treatment reductions in stress reactivity and stress-induced alcohol craving.*

Specific Aim 2: To assess changes in alcohol drinking (drinks per day, heavy drinking days), stress/anxiety (PSS, HAMA, PCL-5), and alcohol craving (PACS, delay discounting, alcohol demand) during treatment in the natural environmental setting.

Hypothesis 2: *We will observe decreased alcohol drinking, stress/anxiety, and alcohol craving over the course of treatment.*

There is increasing attention and evidence supporting the potential role of the PPARγ system as a novel target in the treatment of AUD. From a translational perspective, this study is timely and likely to advance our understanding of stress as a mechanism of action of pioglitazone effects.

BACKGROUND AND RATIONALE

Public Health Impact of AUD. AUD is 1) the 4th leading cause of death in the US (~88,000 deaths/year), decreasing lifespan by approximately 30 years; 2) a tremendous economic burden on society (~$249 billion/year); and 3) associated with a host of short- and long-term negative consequences including violence, legal problems, morbidity, and family problems[14–16].

AUD and the Central Role of Stress. Persistent excessive drinking is a chronic stressor that 1) shifts brain systems beyond normal homeostatic limits into a state of allostasis; 2) which in turn alters physiological and brain motivational systems central for regulating alcohol use; and 3) has negative impact on autoimmune and inflammatory responses that can in turn influence alcohol use[17–21]. Whereas initial drinking is influenced by the positive reinforcing effects of alcohol and impulsivity, continued drinking adopts compulsive characteristics maintained increasingly by negative reinforcement (e.g., alcohol withdrawal symptoms, stress-related anxiety, alcohol craving) as well as decreased stress-resiliency and cognitive deficits from neuro-inflammatory damage[17,22,23]. Despite the profound negative impact of stress on AUD, *none of currently approved medications for AUD directly target stress or stress-related consequences associated with AUD.*

Novelty and Promise of the PPAR System. PPARs are proteins that act as ligand-activated transcription factors, which is central to their anti-inflammatory actions[24] . Pre-clinical studies highlight the potential of PPAR agonists on various aspects of drug use (e.g., discrimination, self-administration, reinstatement, sensitization) for several drugs of abuse including alcohol[11]. In human studies, expression of PPAR has been shown to be altered among individuals with AUD in genome-wide association studies (GWAS) [25 26] studies. In a recent study by our group, post hoc analyses revealed significantly decreased alcohol use among individuals with concurrent cocaine use disorder (CUD) and AUD receiving the PPARγ agonist pioglitazone, compared to placebo, in a study assessing cocaine craving and white matter integrity.[27].

Currently, only the PPAR gamma (PPARγ) isoform can be targeted in humans. PPARγ is highly expressed in a number of brain regions associated with drug reward[28] and stress response[12,13]. Activation of PPARγ mediates neuroprotective responses against inflammatory damage, which can attenuate drug affects[29– 32]. The PPARγ agonist Pioglitazone (Actos) is FDA-approved for the treatment of diabetes and metabolic disorders. In preclinical studies, pioglitazone protects against 1) alcohol-induced neuronal and cognitive damage in a model of binge-drinking[33]; and 2) neuro-inflammation and neurodevelopmental toxicity in a model of fetal alcohol spectrum disorder[34] . Directly relevant to the current study, *pioglitazone 1) significantly reduced alcohol drinking in rats; and 2) attenuated stress-induced alcohol drinking and alcohol withdrawal symptoms in rats bred to highly prefer alcohol*[12,13].

Multi-Dimensional Assessment of Alcohol-Induced Craving. Self-report measures of drug craving are ubiquitous and often serves as a proxy of motivation to consume drug but have limitations that have likely resulted in equivocal results in human laboratory studies[35]. Behavioral economic measures (i.e., delay discounting, alcohol demand) may help mitigate these issues and are both closely associated with drug use and directly tied to a broader framework of drug addiction. Delay discounting is a relatively stable, trait-like measure that assesses the decrease in value of a reward as a function to delay of its receipt, and also serves as a measure of impulsivity. Delay discounting has an extensive literature revealing associations with virtually every aspect of drug use including initiation, severity, and relapse risk[36–38]. Alcohol demand assesses how much alcohol an individual is willing to consume as a function of increasing price, serving as 1) a robust characterization of the reward value of alcohol and therefore craving; and 2) a proxy of compulsive drug use (i.e., consuming alcohol despite increasing aversive consequences). Drug demand can be easily and quickly assessed through purchasing tasks, which have very strong reliability, predictive validity, and are sensitive to state-dependent changes in demand under conditions of withdrawal, cue exposure, environmental stressors,

and predict alcohol treatment response[39–42]. Delay discounting and alcohol demand are poorly correlated with each other, supporting a multi-dimensional model in which these measures represent distinct aspects of motivation to use alcohol[42–48]. In the current proposal, we will assess changes in both delay discounting and alcohol demand over Study Weeks 0 to 4 and alcohol demand will be used to assess state-dependent changes during the human laboratory stress-reactivity assessment.

METHODS

Research team. The research team will consist of Dr. Yoon (PI) and a number of collaborators from UTHealth and Brown University. Dr. Yoon (PI) is an experienced addiction researcher and an expert in assessing behavioral economic measures in addiction research, which is pertinent to the current project. Drs. Weaver (CNRA Medical Director) and Lin (Addictions Psychiatrist) will provide medical guidance and ensure participant safety over the course of the study. Dr. Lane is Professor and Vice Chair for Research and directs the Behavioral Laboratory at the CNRA with expertise in medication development trials as well as alcohol studies. Dr. Suchting is a biostatistician and data scientist with expertise in experimental design and statistical modeling, including the type of generalized linear modeling that will be utilized by the present research. From Brown University, Dr. Haas-Koffler (Co-I) is serving as a consultant on this project and is an expert in stress and alcohol research[1–7]. Additionally, our group has a history of collaborating with the Laboratory of Biomarkers located in the BBSB for analyzing biological samples, such as salivary cortisol in the current protocol. We will work with Dr. Fries to ensure that samples are collected and analyzed appropriately.

Overall study design. We will utilize a within-subject, repeated measures design. Participants will receive pioglitazone or placebo for four weeks. A summary of the study timeline, procedures, and assessments is shown in the following Table.

Table 1. Outline of Study Timeline, Procedures, and Assessments

Study Week **Procedures and Assessments**	**0**	**1**	**2**	**3**	**4**
Consent and Baseline Screening					
SCID, ASI, KMSK, TLFB, CIWAA, AUDIT	X				
Receive Study Medication (Pioglitazone or Placebo)	X	X	X	X	
Assess Medication Compliance and Adverse Events		X	X	X	X
Human Laboratory Procedures (Specific Aim 1)					
Stress-Reactivity Assessment (Salivary Cortisol, HR, BP, Craving)	X				X
Alcohol Demand, Alcohol Ladder	X				X
Natural Environment Procedures (Specific Aim 2)					
Alcohol Use and Craving (Self-Report, BrAC, ETG, PACS)	X	X	X	X	X
Stress and Anxiety (HAM-A, PSS, BDI-II, PCL-5)	X	X	X	X	X
Delay Discounting, Alcohol Demand	X	X	X	X	X

SCID - Structured Clinical Interview for DSM-5**, ASI** - Addiction Severity Index[49]; KMSK - Kreek-McHugh-Schluger-Kellogg scale[50]; **TLFB** - Timeline Followback[51]; CIWAA - Clinical Institute Withdrawal Assessment for Alcohol[52]; **AUDIT** – Alcohol Use Identification Test[53]; **BrAC** – Breath Alcohol Concentration; **ETG** – Ethyl Glucuronide; **PACS** – Pennsylvania Alcohol Craving Scale[54]; **HR** – Heart Rate; **BP** – Blood Pressure; **HAM-A** – Hamilton Anxiety Rating Scale[55]; **PSS** – Perceived Stress Scale[56]; **PCL-5** - PTSD Checklist for DSM-5; **BDI-II** – Beck Depression Inventory - II**.**

Participants, Recruitment, and Setting. Participants **(N=20**) will consist of non-treatment and treatment-seeking individuals diagnosed with AUD (DSM-5) between 21 to 40 years old and fluent in English; past month excessive alcohol use (>7 drinks/week for woman, >14 drinks/week for men, >3 drinks/occasion for women>4 drinks/occasion for men)[14]; baseline HAM-A or PSS Score indicative of mild to moderate anxiety (score 8 to 23) or moderate stress (score 14 to 26), respectively; and increase in alcohol craving following the

baseline stress reactivity assessment. Individuals will be excluded for exhibiting severe scores on the HAM-A, PSS, BDI-II, or PTSD checklist (PCL-5) at the discretion of the admitting physician (Dr. Weaver); physical dependence on alcohol; greater than mild substance use disorder on drugs other than alcohol, nicotine, and marijuana; contraindications for taking pioglitazone; medical conditions contraindicating pioglitazone pharmacotherapy or taking contraindicated medications; be pregnant, nursing, or planning on becoming pregnant during the course of the study; have any other illness, condition, or use of medications, which in the opinion of the PI and/or admitting physician would preclude safe and/or successful completion of the study. Recruitment strategies will include local advertising in print media, public service announcements on radio, and referrals to CNRA in the Houston metropolitan area.

Baseline Screening. Participants will receive a comprehensive medical and psychiatric evaluation including: a medical-history questionnaire, physical examination, laboratory chemistries (e.g., blood chemistry screen, complete blood count, urinalysis and urine pregnancy test), and ECG. Clinicians will conduct the **SCID**, the **ASI** ASI[49], the **KMSK**[50] assessment of lifetime substance use interview, and **TLFB**[51]. Alcohol dependence will be assessed using the SCID and the **CIWAA**[52].

Alcohol-Related Measures. Assessment of alcohol use will include self-report **(TLFB)**, **BrAC** (Alco-Sensor FST, Intoximeters, Inc., Saint Louis, MO). Severity of alcohol use will be assessed using the **AUDIT**[53]. **Motivation to quit** will be assessed using the Alcohol Ladder questionnaire. **Alcohol craving** will be assessed using a 5-item questionnaire[57]. ETG will be assessed using dipcards with a 300 ng/ml cutoff. ETG dipcards show good agreement with traditional immunoassays[58–60].

Behavioral Economic Measures. Alcohol demand will be assessed via the **Brief Assessment of Alcohol Demand** (BAAD)[42], a 3-item questionnaire measuring the three most common indices of alcohol demand. **Delay discounting** will be assessed for both money and alcohol using a computerized task at weekly clinic visits developed by Dr. Yoon (PI)[61,62,71,63–70].

Stress and Anxiety Measures. Stress and anxiety levels will be assessed using the **HAM-A**[55], **PSS**[56], **BDI-II**, and the **PCL-5**. Established norms will be used for the HAM-A and PSS to assess mild to moderate stress and anxiety and screen out for cases of severe stress and anxiety.

Stress Reactivity Measures. HR and BP will be assessed using standard laboratory equipment. Saliva samples will be collected in swabs using the Cortisol-Salivette® system (Sarstedt) and measured using the Cortisol ELISA Kit (Enzo Life Sciences), per manufacture instructions.

Study Medication. Pioglitazone (Actos®, Takeda Pharmaceuticals U.S.A., Inc.) is FDA-approved for the treatment of diabetes mellitus type 2; has anti-inflammatory, neuroprotective, antioxidative, and anti-excitotoxic properties[72]; and extensive research has shown it to be safe in human patient populations[73,74] . The medication schedule is based on previous work from our group showing that a daily dose of 45mg was associated with acceptable levels of tolerability, safety, and compliance in a study assessing the effects of pioglitazone on individuals with CUD and AUD[27]. Our pilot trial used a conservative 2-week dose titration schedule (i.e., Week 1: 15 mg/d; Week 2: 30 mg/d), with no adverse effects reported or observed. For the current trial we will follow recommended adult initial dosing at 30 mg/d to reach maintenance dose of 45 mg/d by week 2, which is within standard titration parameters as per the investigator's brochure. Thorough screening of all subjects including history and physical examination, serum chemistry and hematology, EKG, urinalysis and Urine Drug Screen as well as structured psychiatric interviews will be completed on all subjects. Exclusion criteria include subjects at greatest risk of side effects of pioglitazone, including those with congestive heart failure, significant liver disease, edema, and diabetes, as well as risks of alcohol use (pregnant

women). Monitoring of weight to capture potential fluid retention will occur at each visit along with an adverse event assessment to include assessment for edema. Blood will be drawn at the end of the study for liver function testing. Female subjects must agree to use an effective barrier method of birth control and urine pregnancy tests will be performed at each visit to minimize risk of pregnancy.

Medication Compliance. One week supplies of the study medication will be dispensed with a Medication Event Monitoring System (MEMS) cap to record the number of bottle openings and date/time of each opening. Riboflavin will be added to medication capsules and urine ultraviolet fluorescent tests will be conducted at weekly clinic visits to assess compliance. Participants will receive $10 at each clinic visit in which results from self-report, MEMS, and riboflavin are all consistent with medication compliance.

Human Laboratory Procedures (Specific Aim 1). On Study Weeks 0 and 4, participants will engage in the cold-pressor task (CPT). The CPT is widely used as a stress-inducer in human laboratory studies and elicits moderate activation of the sympathetic nervous system and limited activation of the HPA-axis, which are two major stress systems in the body[75–78]. HPA-axis activation during the CPT can be increased by incorporating a social evaluative component. Specifically, a study member of the opposite sex of the study participant will dress in a white labcoat and be physically present during the CPT. Additionally, participants will be videotaped and informed that their facial expressions will be assessed during the CPT. The addition of the social component during the CPT has been demonstrated to selectively activate the HPA-axis and significantly increase salivary cortisol levels[77]. During the CPT, participants will submerge their dominant arm in an ice-water bath for up to 2 minutes. Stress levels will be assessed using subjective and physiological measures HR, BP, and salivary cortisol consistent with previous studies from our group[79,80]. Alcohol craving will be assessed using a 4-item questionnaire[57].

Table 2. Time Course for Assessing Stress-Induced Relapse Risk

Time (pm)	Study Procedures
3:00	Assess Craving, BAAD, Stress, Cortisol, HR, BP
3:15	Cold Pressor Task
3:20	Assess Craving, BAAD, Stress, HR, BP
3:45	Assess Craving, BAAD, Stress, Cortisol, HR, BP

Natural Environment Procedures (Specific Aim 2). At Study Week 0, all participants will receive a pamphlet copy of the "Rethinking Drinking: Alcohol and Your Health" providing research-based information related to alcohol use (RethinkingDrinking.niaaa.nih.gov). Research staff will go over the pamphlet's information with the participant at the initial visit and subsequent visits as necessary. At each study visit, participants will receive study medication for the week and assessed for adverse events and medication compliance. At each visit, participants will also be assessed for recent alcohol use (self-report, ETG, BrAC), alcohol craving (PACS), and stress/anxiety (HAM-A, PSS). At the end of the study, participants will be referred to local alcohol treatment services as needed.

Participant Payment & Compensation. Participants will receive be able to receive $300 for completing the study ($50 for completing baseline screening; $25 for each visit ($100); $25 for stress reactivity assessments ($50); and $100 completion bonus). Additionally, participants will potentially receive $10/week (up to $40) for demonstrating medication compliance. Participants will also receive parking/bus fare compensation at each visit worth $5.

DATA ANALYSIS PLAN

General Data Analytic Strategy. Analyses will proceed in parallel using frequentist and Bayesian statistical inference. Frequentist analyses will examine relationships between predictors and outcomes using traditional measures of effect size and statistical significance (i.e., regression coefficients, standard errors,

confidence intervals, and *p*-values), while Bayesian analyses will measure uncertainty in model parameter estimates via posterior distributions and directly evaluate the probability of the alternative hypothesis.

Specific Aim 1: assess pre- to post-treatment reductions in stress reactivity (self-report, salivary cortisol, heart rate, blood pressure) and stress-induced alcohol craving (self-report, alcohol demand) in the human laboratory setting.
Hypothesis 1: We will observe pre- to post- treatment reductions in stress reactivity and stress-induced alcohol craving. Generalized linear mixed modeling will be used to evaluate each outcome as a function of time (pre-test and post-test) in unique models.

Specific Aim 2: Demonstrate reduced alcohol drinking (drinks per day, heavy drinking days), stress/anxiety (PSS, HAMA, PCL-5), and alcohol craving (PACS, delay discounting, alcohol demand) during treatment in the natural environmental setting.
Hypothesis 2: We will observe decreased alcohol drinking, stress/anxiety, and alcohol craving over the course of treatment. Generalized linear mixed modeling will be used to evaluate each outcome as a function of time in unique models. Alcohol consumption will be modeled via drinks per drinking day, such that the number of positive drinking episodes a participant experienced in a week will be included as a count outcome (i.e., Poisson-distributed) and the number of drinking days in a week will be controlled for as an off-set covariate.

Power/Sample Size Considerations. The frequentist framework using null hypothesis statistical testing can provide some understanding of changes over time for each outcome. However, in evaluating a small within-subjects pilot trial of N = 20 participants, Bayesian statistical inference provides a superior framework for deriving evidence regarding the magnitude and direction of change over time for each model. The posterior probability of each effect would then be useful to inform effect sizes for power calculations and/or prior distributions in future research. As due diligence, however, a conventional frequentist power analysis follows. For the current proposal, power estimates are calculated using G*Power 3.1.9.2 and focus on reduction in alcohol consumption. Assuming alpha = 0.05 (one-tailed), an average baseline rate of 10.5 drinks/week across all participants (averaged between 14 and 7 drinks/week for male and female participants, respectively), a sample size of N = 20 participants provides 80% power to detect a 20% decrease in alcohol consumption over five weeks.

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