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# Effects of Ibudilast on Central and Peripheral Markers of Inflammation in Alcohol Use Disorder: A Randomized Clinical Trial

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## Abstract

Ibudilast, a neuroimmune modulator, shows promise as a pharmacotherapy for alcohol use disorder (AUD). *In vivo* administration of ibudilast reduces the expression of pro-inflammatory cytokines in animal models, but its effects on markers of inflammation in humans are unknown. This preliminary study examined the effect of ibudilast on peripheral and potential central markers of inflammation in individuals with AUD. This study also explored the predictive relationship of neurometabolite markers with subsequent drinking in the trial. Non-treatment-seeking individuals with an AUD (n=52) were randomized to receive oral ibudilast (n=24) or placebo (n=28) for two-weeks. Plasma levels of peripheral inflammatory markers were measured at baseline, and after 1 and 2 weeks of medication. At study mid-point, proton magnetic resonance spectroscopy (MRS) was performed to measure potential neurometabolite markers of inflammation: choline-

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ENG, EDL, CJE, MRI and LAR conceived and designed the study. ENG, EB, and SN collected the experimental data. ENG, JO, JA, and MRI processed and analyzed the results. ENG and LAR drafted the manuscript with all authors contributing to the interpretation of the results. All authors reviewed the results and approved the final version of the manuscript.

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compounds (Cho), *myo*-inositol (MI), and creatine+phosphocreatine (Cr) in frontal and cingulate cortices from 43 participants (ibudilast: n=20; placebo: n=23). The treatment groups were compared on peripheral and central markers. Ibudilast-treated participants had lower Cho in superior frontal white matter and nominally lower MI in pregenual anterior cingulate cortex. Ibudilast-treated participants had nominally lower CRP levels at visit 2 and nominally lower TNF-α/IL-10 ratios, relative to placebo. CRP and Cho levels were correlated, controlling for medication. Superior frontal white matter Cho predicted drinking in the following week. Micro-longitudinal ibudilast treatment may induce peripheral and putative central anti-inflammatory responses in patients with AUD. The neurometabolite responses may be associated with reduction in drinking, suggesting an anti-inflammatory component to the therapeutic action of ibudilast.

### Keywords

Ibudilast; Alcohol Use Disorder; Cytokine; Magnetic Resonance Spectroscopy; Choline; Anti-Inflammatory

## Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder with a significant public health impact. Treatment rates for AUD remain low despite the substantial negative consequences associated with this disorder[1]. The 2019 National Survey on Drug Use and Health (NSDUH) found that only 1.6% of US adults with a past-year diagnosis of AUD received an FDA-approved medication to treat this problem[2]. Moreover, FDA-approved AUD pharmacotherapies are only modestly effective[3], with number needed to treat ranging from 7 to144 patients across medications and studies[4]. Thus, there is an urgent need for the development of novel treatments, especially ones with novel targets[5], including the immune system[6].

The neuroimmune system has been implicated in the development and maintenance of AUD[7]. In animal models, voluntary ethanol consumption and ethanol withdrawal increase inflammatory cytokines and chemokines in the brain and in periphery[8–11]. However, other preclinical work has found decreases or no differences in cytokine and chemokines depending on the animal model and method of administration (reviewed in[12]). Preclinical work suggests that neuroinflammatory states induced by chronic alcohol use heighten motivation for intake, enhance alcohol-related reward, and contribute to substance-related cognitive impairments and depression-like behavior[13–17]. In humans, post-mortem brain tissue of individuals with AUD shows evidence of upregulation of proinflammatory gene expression[18–20]. In clinical samples, levels of peripheral pro-inflammatory cytokines are higher in individuals with AUD than in controls[21,22]. Yet, the degree to which AUD-related inflammation can be reliably detected in the living human brain remains unclear[23,24].

Proton magnetic resonance spectroscopy (MRS) allows for the non-invasive detection of neurometabolites *in vivo*. Several neurometabolites are thought to serve as markers of neuroinflammation[25,26]. These include *myo*-inositol (MI), a glial marker primarily found in gray matter; choline-containing compounds (Cho), a marker for cell membrane metabolism and turnover primarily found in white matter; and creatine+phosphocreatine (Cr), a marker

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for energy reserves of neurons and glia[25]. Elevations in MI, Cho, and Cr have been found across a range of neuroinflammatory disorders, including multiple sclerosis, Human Immunodeficiency virus, Hepatitis C, Alzheimer's disease, and Parkinson's disease[25]. Levels of MI and Cho have been shown to correlate with peripheral markers of inflammation in healthy individuals across the lifespan[26]. Cho levels have also been shown to correlate with the volume of perivascular spaces[27], which are thought to be markers of inflammation. Further, *N*-acetyl-compounds (NAA) are widely regarded as a marker of neuronal integrity and metabolism[28], with some evidence for anti-inflammatory action[29]. A substantial body of literature implicates frontocortical circuitry in the phenomenology of AUD including superior frontal cortex, superior frontal white matter (SFWM), and anterior cingulate cortex (ACC)[30–34]. Therefore, it is plausible that AUD-related neuroinflammation occurs in these structures, which might be targeted by neuroimmune pharmacotherapies.

One such neuroimmune pharmacotherapy is ibudilast, a preferential inhibitor of PDE3A, −4, −10A, and −11A[35] and an allosteric inhibitor of macrophage migration inhibitory factor (MIF)[36]. PDEs are enzymes which regulate the intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP)[37]. PDEs modulate the cAMP protein kinase pathway, which has been implicated in the regulation of response to acute and chronic exposure to alcohol[38]. Both PDE4 and MIF are involved in neuroinflammatory processes through the regulation of inflammatory responses in microglia[39,40], and PDE4B expression is upregulated after chronic alcohol exposure[41]

Recent evidence suggests that ibudilast might show promise as a pharmacotherapy for AUD[42,43]. In animal models of AUD, administration of ibudilast has been found to reduce drinking and relapse, and, preferentially reduced drinking in dependent as compared with non-dependent mice[44]. In humans we have found that ibudilast reduced tonic craving and improved mood reactivity to stress and alcohol cue exposure compared to placebo[42]. In addition, in a micro-longitudinal clinical trial, ibudilast decreased heavy drinking and attenuated alcohol cue-elicited ventral striatal activation, which was predictive of subsequent drinking[43].

The mechanisms by which ibudilast alters drinking outcomes are not known, although attenuation of alcohol-related inflammation is implicated. *In vitro*, ibudilast suppressed pro-inflammatory cytokines (interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α), and enhanced the production of IL-10, an anti-inflammatory cytokine in activated microglia[45]. In preclinical models of neurological pathologies, ibudilast reduced IL-1β, IL-6, and TNF-α expression[46,47]. However, in humans, there has been limited research on the neuroimmune effects of ibudilast, and no studies to our knowledge have been conducted in samples of individuals with AUD. In patients with methamphetamine use disorder, ibudilast reduced methamphetamine-induced increases in levels of adhesion molecule inflammatory markers[48].

Despite the promise of ibudilast as an AUD pharmacotherapy, it is not known whether ibudilast modulates immune processes as a potential mechanism for the reduction of alcohol drinking. In this preliminary analysis of a two-week trial of ibudilast in non-treatment-seeking individuals, we examined the effect of ibudilast on peripheral markers of systemic

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inflammation, as well as possible central markers of neuroinflammation. Blood was sampled to assess levels of C-reactive protein (CRP), IL-6, IL-8, IL-10, interferon gamma (IFN-γ), TNF-α, and the TNF-α/IL-10 ratio, reflecting the balance of pro-inflammatory and anti-inflammatory cytokines. MRS was performed to assess *in vivo* markers thought to measure neuroinflammation (Cho, MI, and Cr) and neuronal injury (NAA), in cerebral cortex and white matter[25,26]. We hypothesized that ibudilast would decrease peripheral markers of inflammation and increase peripheral markers of anti-inflammation, and that participants treated with ibudilast would show lower levels of putative pro-inflammatory neurometabolites and higher levels of anti-inflammatory NAA compared to placebo. This study also explored the predictive relationship of neurometabolite markers and subsequent drinking in the trial.

## Materials and Methods

### Participants

Fifty-two non-treatment-seeking individuals with AUD were enrolled and randomized to receive oral ibudilast (n=24) or matched placebo (n= 28) for two-weeks (see Consort Diagram, Figure 1)[43]. Eligible participants were between 21 and 50 years of age, met criteria for a current DSM-5 diagnosis of AUD, mild-to-severe, and drank >14 drinks/week for males and >7 drinks/week for females. Exclusion criteria were: currently receiving or seeking treatment for AUD; past year DSM-5 diagnosis of a substance use disorder (other than AUD or tobacco use disorder); lifetime diagnosis of schizophrenia, bipolar disorder, or any psychotic disorder; nonremovable ferromagnetic objects in body; claustrophobia; and serious head injury or prolonged period of unconsciousness (>30 min). Participants were excluded if they had a medical condition that had a potential to interfere with safe participation and if they reported recent use of medications contraindicated with ibudilast. Female participants of a childbearing age had to be practicing effective contraception and could not be pregnant or nursing. Participants were randomized in the study between July 2018 and March 2020 (see Grodin et al., 2021 for full study details).

### Study Design

This was a micro-longitudinal clinical study (ClinicalTrials.gov identifier: NCT03489850). Participants completed three in-person visits, during which they provided blood samples and completed questionnaires at baseline (Study Day 1), study mid-point (Study Day 2), and study-endpoint (Study Day 3, final follow-up visit). On Study Day 2, participants underwent MRS scans of the brain. Participants also completed daily diary assessments to report on their past day drinking, mood, and craving (see Grodin et al., 2021). Participants were required to have a breath alcohol concentration of 0.00 g/dl at each in-person visit. This trial was approved by the Institutional Review Board of the University of California, Los Angeles. All study participants provided written informed consent after discussing the study medication with the study physician (KM).

Participants were randomized to receive 50 mg b.i.d. of ibudilast or placebo, supplied by MediciNova, Inc. A stratified randomization list was developed by a statistician and was based on sex and withdrawal-related dysphoria, a measure of AUD severity. The UCLA

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Research pharmacy prepared both test medications in blister packs, which were dispensed on the randomization study visit. Participants, providers, and research staff remained blind to medication assignment throughout the study. Ibudilast was titrated as follows: 20 mg b.i.d. during days 1–2 and 50 mg b.i.d. during days 3–14. Medication compliance was monitored through pill counts at the midpoint and final study visits, and through self-report in the daily diary assessments.

### Daily Diary Assessments

Participants completed daily diary assessments, reporting on their past-day alcohol use, mood, and craving (primary results reported in Grodin et al., 2021). Alcohol use was assessed by asking the number of standard drinks that were consumed yesterday. Non-standard alcohol use was assessed by asking the type of non-standard alcohol consumed (e.g., malt liquor) and the number of drinks consumed. Non-standard drinks were converted to standard drinks and the number of drinks consumed were totaled.

### Assessment of peripheral inflammation

Blood samples were collected on Study Days 1, 2, and 3 by venipuncture into EDTA tubes, placed on ice, centrifuged for acquisition of plasma, and stored at 80°C for batch testing. CRP levels were determined utilizing the Human CRP Quantikine ELISA (R&D Systems) according to the manufacturer’s protocol with a lower limit of detection of 0.2 mg/L. Samples were assayed in duplicate. Intra- and inter-assay precision of all tests was <6.1%. For the small proportion (2%, n=3) of samples with CRP concentrations above the upper limited of the standard curve (>25 mg/L) a value of 25 mg/L was assigned. For the small proportion (9%, n=13) of samples with CRP levels below the limit of detection (0.2 mg/L), a value of 0.2 mg/L was assigned.

Plasma levels of TNF-α, IL-6, IL-8, IL-10 and IFN-γ were evaluated using the Meso Scale Discovery (MSD) MULTI-SPOT Assay System (Rockville, MD[49]). Plasma samples were assayed in duplicate on a custom 5-plex from the Proinflammatory Panel 1 Human Kit. Briefly, blood samples were collected in EDTA tubes, and processed at 4°C to plasma aliquots. Plasma aliquots were stored at −80°C until assayed in a single batch. Assays were performed according to the manufacturer’s protocol. ECL signals were measured on the MESO QuickPlex SQ 120 instrument (Rockville, MD), and the DISCOVERY WORKBENCH software (Rockville, MD) was used to generate a 4-parameter logistic fit curve. The mean intra-assay coefficient of variation (CV) for IFN-γ was <6%, and the mean inter-assay CV <12.2%, with similar CV’s for other inflammatory markers. Levels of IL-8, IFN-γ, and TNF-α were detectable in all subjects. For IL-6, 3.5% of the samples (n=5) were below the level of detection (0.2 pg/mL) and were assigned a value of 0.2 pg/mL. For IL-10, 0.7% of the samples (n=1) were below the level of detection (0.1 pg/mL) and were assigned a value of 0.1 pg/mL.

### Neuroimaging Protocol

**Magnetic Resonance Acquisition—**Participants were scanned at Study Day 2 (mid-point) on a 3.0 Tesla Siemens Prisma Scanner (Siemens Medical Solutions USA, Inc.; Malvern, PA). Anatomical T1 images were obtained through a magnetization-prepared rapid

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gradient-echo (MPRAGE) sequence (TR = 2,530 ms, TE = 1.74 ms, time to inversion = 1,260 ms, flip angle = 7°, voxel size: 1 $mm^3$, FOV = 250×250 $mm^2$, ~6.2 minutes). This MPRAGE and coronal-oblique and axial-oblique resliced copies were used to prescribe MRS.

The MRS acquisition and post-processing followed methods that have been described previously[50,51]. Briefly, 2-dimensional water-suppressed proton magnetic resonance spectroscopic imaging was acquired with a stimulated-echo acquisition mode (STEAM) pulse-sequence with TR/TE/TM = 2000/20/10 ms, voxel dimensions 10×10×10 $mm^3$, and 4 excitations. The field of view was 160×160 mm2 and the slab thickness was 10 mm. A non-water-suppressed acquisition using 1 excitation was acquired from an identical volumetric prescription, and the non-water-suppressed data were used for offline quality control, and quantitation of metabolites. The prescription consisted of a coronal-oblique 16×16 matrix (voxel-array) oriented tangent to the dorsum of the corpus callosum as seen in the sagittal plane. The 8×8 subarray in the center of the slab constituted the "excitation box", resulting in an excitation volume of 80×80×10 $mm^3$, from which usable magnetic resonance spectra were recorded. Rostro-caudally this box extended approximately from pregenual anterior cingulate to premotor cortex. Lateral-mesially it straddled the longitudinal fissure symmetrically and extended to lateral cortices (e.g., middle frontal cortex). See Figure 2 for visualization of the voxel array and sample spectrum.

This study was performed using the standard Siemens product two-dimensional STEAM-CSI pulse-sequence with 16×16 elliptical phase-encoding. Spatial reconstruction was performed with the standard Siemens software. The reconstruction used a Hamming filter with a filter factor of 50%. Siemens documentation uses two parameters (Λ and K) to characterize the point spread function (PSF) for this reconstruction. Λ specifies the PSF full-width at half-maximum (FWHM). K quantifies the outside-of-voxel contamination as the ratio of outside-of-FWHM to inside-of-FWHM signal intensity. For the parameters used in this reconstruction, Siemens documentation reports Λ = 1.49, and K = 1.64 and that the effective voxel size is approximately 1.5 times the nominal voxel size, or 1.5 cc.

**Magnetic Resonance Spectroscopy Post-Processing—**All postscan processing was performed by staff blinded to medication condition. MPRAGE images were segmented into gray matter, white matter, and CSF subvolumes using FSL FAST. Further, each MPRAGE was parcellated bilaterally into regional volumes-of-interest (VOIs) using FreeSurfer. These included pregenual anterior cingulate cortex (pACC), superior frontal cortex (SFC), and superior frontal white matter (SFWM). Each tissue subvolume and VOI was converted into a binary mask and reconstructed into the native space of each MRS voxel using SVFit2016[52].

Operations performed by SVFit2016 included time-domain filtering and non-linear least-squares spectral fitting to determine neurometabolite levels for each magnetic resonance spectroscopic imaging voxel within the excitation box (exclusive of box edges). SVFit2016 was written in the Interactive Data Language and uses the Levenberg–Marquardt implementation of the Gauss-Newton method to fit spectra in the frequency domain. The specific fitting routine is a modified version of MPFIT[53] (http://purl.com/net/mpfit). Fits for non-water-suppressed spectral arrays used a model spectrum that included only a single

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water signal. Fits for water-suppressed spectra included models of spectra for lactate, *N*-acetylaspartate, *N*-acetyl-aspartyl-glutamate, glutamate, glutamine, $\gamma$-aminobutyric acid, creatine, phosphocreatine, choline-compounds, inositol compounds, numerous low-level neurometabolites, residual water, lipids, and macromolecules. Model spectra were simulated in Versatile Simulation, Pulses and Analysis (VESPA) software[54–56] (https://scion.duhs.duke.edu/vespa/project). Following fitting, the *N*-acetylaspartate and *N*-acetyl-aspartyl-glutamate signals were summed to form total *N*-acetylaspartate (NAA). Similarly, creatine and phosphocreatine were summed to total creatine (Cr). Fit quality for all spectra was reviewed by two experts (JRA, JON). Poor fits determined by visual inspection were resubmitted for fitting with different starting estimates of various parameters. Voxel spectra that showed poor fit quality after multiple retries were not included in further analyses. Spectra with signal-to-noise ratio <5 in the Cr spectral region were excluded, as were spectra with voxel static magnetic field inhomogeneity >0.1 parts-per-million.

## Statistical Analysis

All statistical analyses were conducted in SAS 9.4. Inflammatory marker levels were not normally distributed (skewness range 1.50–6.80) and were therefore log-transformed prior to statistical analysis.

A series of linear regression models were tested using PROC GLM to evaluate the effect of medication (i.e., ibudilast vs. placebo) on MRS neurometabolite concentrations (Cho, MI, Cr, NAA) for three regions of interest (ROIs): pregenual anterior cingulate cortex (pACC) superior frontal cortex white matter (SFWM), and superior frontal cortex (SFC). ROIs were calculated as the average of the left- and right-hemisphere regions. Age, sex, and smoking status were included as covariates. Analyses were adjusted for multiple comparisons, and the significant p-value was set at 0.0125 (i.e., 0.05/4 for the 4 metabolites). We did not divide by the number of regions as we had *a priori* evidence for effects of AUD in each region. Uncorrected results for the unilateral regions are reported in the Supplement. Effect sizes were calculated as $\eta_p^2$.

Inflammatory marker analyses were conducted in a multilevel framework using PROC MIXED, where the effect of medication (ibudilast, placebo), time (Study Day 2, Study Day 3), and their interaction were examined. Age, sex, smoking status, body mass index, drinking prior to randomization (drinks per drinking day), and baseline (Study Day 1) inflammatory marker levels were included as covariates.

To evaluate the relationship between neural and peripheral markers, partial correlations between MRS metabolites and peripheral inflammatory marker levels at Study Day 2 were conducted, controlling for medication.

Finally, to examine the clinical relevance of these markers, exploratory linear regression models were tested to evaluate the effects of medication, MRS metabolite levels, and their interactions on the number of drinks per drinking day in the week following the neuroimaging scan. Age, sex, smoking status, and baseline number of drinks per drinking day were included as covariates.

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# Results

## Participants

Fifty-two participants were randomized to receive ibudilast or placebo. Of those randomized, two did not complete the trial (n=1/group). All 52 participants provided blood samples for baseline levels of inflammatory markers on Study Day 1. Forty-seven participants provided blood samples on Study Day 2 (ibudilast: n=22; placebo: n=25) and 46 participants provided blood samples on Study Day 3 (ibudilast: n=23; placebo: n=23). Of the 45 participants who completed the neuroimaging scan, 43 had usable MRS data (ibudilast: n=20; placebo: n=23; see Figure 1) The groups did not differ on demographic or clinical characteristics or on their baseline levels of inflammatory markers (see Table 1).

## MRS Metabolites

Individuals treated with ibudilast had significantly lower Cho levels in mean SFWM ($F(1,42) = 6.88$, $p = 0.0125$; $\eta_p^2 = 0.15$; Figure 3A). The ibudilast group had trend-level lower MI levels in the mean pACC ($F(1,31) = 3.06$, $p = 0.09$; $\eta_p^2 = 0.07$; Figure 3B). There were no significant effects of age, sex, or smoking status on neurometabolite levels. The uncorrected unilateral analyses found that individuals treated with ibudilast also had lower Cr in left pACC ($F(1,31) = 4.63$, $p = 0.04$. $\eta_p^2 = 0.15$), but higher Cr in the right SFC ($F(1,39) = 4.61$, $p = 0.03$, $\eta_p^2 = 0.13$); and higher NAA in right SFC ($F(1,39 = 6.39$, $p = 0.02$, $\eta_p^2 = 0.15$; see Supplementary Table S1).

## Inflammatory Markers

There was a trend-level interaction between medication and time for CRP ($F(1,40) = 3.50$, $p = 0.07$; Figure 4A), such that for individuals treated with ibudilast, CRP levels decreased from time 1 to time 2, while individuals treated with placebo had increases in their CRP levels from time 1 to time 2. At trend level, ibudilast-treated participants also had lower TNF-α/IL-10 ratios across timepoints relative to placebo ($F(1,39) = 3.68$, $p = 0.06$; Figure 4B). There was a main effect of medication on IL-8 across timepoints after accounting for baseline levels ($F(1,40) = 7.45$, $p = 0.009$; Figure 4C); however, this effect appears to be driven by an unexpected decrease in IL-8 in the placebo group. There were no significant effects of medication or medication by time interactions on IL-6, IL-10, IFN-γ or TNF-α levels (See Supplementary Table S2 and Supplementary Table S3).

## Association Between CNS and Peripheral Markers

Log CRP levels at Study Day 2 and Cho in the mean SFWM levels were correlated, controlling for medication ($r = 0.32$, $p = 0.04$, $n = 42$). Log IL-8 levels at Study Day 2 and mean pACC MI levels were negatively correlated ($r = -0.33$, $p = 0.04$, $n = 40$).

## Clinical Prediction

There was a significant interaction between medication and Cho levels in the mean SFWM in predicting drinks per drinking day in the week following the scan ($F(1,42) = 5.05$, $p = 0.03$; $\eta_p^2 = 0.06$). Specifically, in the ibudilast group, there was a positive relationship

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between Cho levels and the number of drinks per day, such that those with lower levels of Cho had fewer drinks per drinking day and those with higher Cho had more drinks per drinking day. There was no relationship between Cho and drinking in the placebo group (see Figure 5). There was no significant interaction between medication and MI levels on drinking in the week following the scan ($p = 0.49$).

## Discussion

This preliminary study examined the effects of ibudilast, versus placebo, on putative central and peripheral markers of inflammation in individuals with AUD. In support of our hypothesis, participants treated with ibudilast had significantly lower levels of neurometabolite markers in the SFWM and nominally lower levels in the pACC, relative to placebo-treated participants. Ibudilast-treated participants had lower CRP levels and TNF-α/IL-10 ratios, albeit at trend level, relative to placebo. Exploratory analyses found that Cho levels in the SFWM were predictive of subsequent drinking in the week following the scan in the ibudilast group. Together, these preliminary results suggest that ibudilast may work through a neuroimmune modulation mechanism to reduce drinking in individuals with AUD.

Consistent with the hypothesis that ibudilast reduces neuroinflammation, ibudilast-treated participants had lower levels of proposed neurometabolite markers of inflammation. Specifically, in the SFWM, individuals treated with ibudilast had significantly lower levels of Cho, a marker for cell membrane metabolism and cellular turnover[25]. Cho concentrations are higher in glia relative to neurons and elevations in Cho may reflect glial activation and/or acute cell membrane injury, reflective of neuroinflammation[25]. *In vitro*, ibudilast dose-dependently reduces microglial activation[45]; *in vivo*, ibudilast reduces white matter damage[57]. The literature surrounding Cho levels in AUD has been mixed. White matter and thalamic Cho has been shown to positively correlate with alcohol consumption in social drinkers[58] and chronic heavy drinkers[59], such that higher Cho levels were indicative of more drinking. Binge drinking and longer length of AUD have also been shown to positively correlate with higher thalamic Cho levels in individuals with AUD[60]. Animal models of AUD and binge drinking have also found higher Cho levels using MRS[61,62]. However, lower prefrontal, thalamic, and cerebellar Cho levels have also been reported in individuals with AUD[60,63,64]. Differences in participant characteristics, including treatment-seeking status, non-abstinence, and binge drinking, may contribute to these mixed findings. In the present study all participants were non-treatment-seeking and the majority continued drinking throughout the study; although the ibudilast group reduced their heavy drinking relative to placebo[43]. Therefore, the lower Choline levels in the ibudilast group may reflect ibudilast-associated decreases in heavy drinking, consistent with the association between subsequent drinks per drinking day and Choline levels in this group.

Participants treated with ibudilast also had lower MI levels in the pACC, relative to placebo. MI is an osmolyte which is primarily found in glial cells; elevations in MI are thought to reflect activated glial cells which have enlarged cell volumes[25]. Several studies have reported higher MI levels in individuals with AUD relative to controls (reviewed in[65]). Treatment-seeking individuals with AUD show elevated MI levels in early abstinence, potentially due to alcohol-induced hyperosmolarity which may cause MI accumulation[66].

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The lower MI levels seen in ibudilast-treated individuals may reflect osmolar stability or a reduction in the activation of glial cells. Therefore, ibudilast may be working in an anti-inflammatory manner to reduce microglial activation in individuals with AUD.

Consistent with the neuroimmune hypothesis of AUD, participants generally showed elevations in peripheral markers of inflammation at baseline compared to levels reported in previous studies of healthy controls[67,68]. Individuals treated with ibudilast had lower TNF-α/IL-10 ratios, and lower CRP levels at the end of the study, albeit at trend level. In cell culture, ibudilast suppressed TNF-α production[69]. In patients with multiple sclerosis, treatment with ibudilast downregulated TNF-α and upregulated IL-10 mRNA in blood CD4+ cells[70], consistent with the ratio results of the current study. For the substance use disorder indication, ibudilast attenuated methamphetamine-induced levels of pro-inflammatory adhesion molecules, sICAM-1 and sVCAM-1[48]. Elevated levels of circulating TNF-α and CRP have been reported in individuals with AUD and chronic heavy drinkers[22,71,72]. Therefore, it is plausible that ibudilast acts in an anti-inflammatory manner, reducing peripheral markers of inflammation. Of note, these ibudilast-associated decreases were not consistent between all peripheral immune markers, in line with the mixed findings of preclinical and clinical neuroimmune AUD studies[12].

While there was a main effect of medication on IL-8 levels, it appears that this effect was driven by an unexpected decrease in IL-8 levels in the placebo group. We anticipated that cytokine and chemokine levels in the placebo group would stay relatively stable over the course of the two-week study and levels in the ibudilast group would modulate. Given that these findings appear to be influenced by the decrease in the placebo group, conclusions about the effects of ibudilast on IL-8 levels should be made with caution and pending replication. The effects of treatment on circulating cytokine profiles of individuals with AUD remains emergent and additional studies with larger samples are needed to fully elucidate these profiles.

In addition to testing proof-of-mechanism, exploratory clinical prediction analyses found an association between Cho levels and subsequent drinking in the ibudilast treated group. Individuals treated with ibudilast who had the lowest Cho levels in the SFWM also had the fewest number of drinks per drinking day in the week following the MRS scan, controlling for baseline drinking levels. In rodents, binge ethanol exposure increased Cho levels and Cho levels were associated with *in vivo* ethanol levels[62]. In non-abstinent social drinkers, Cho levels were also positively associated with alcohol consumption[58]. In the current study, this predictive relationship was only present in the ibudilast group and not in the placebo group. This indicates that Cho levels are not merely reflective of current drinking, as in that case we would expect similar associations between drinking and Cho in the placebo group. Therefore, this finding indicates that for individuals treated with ibudilast, modulation of Cho levels, potentially reflecting modulation of neuroinflammation, was related to subsequent drinking. Additionally, CRP levels on Study Day 2 were positively correlated with SFWM Cho levels. This is important, as peripheral inflammatory markers are more clinically obtainable than collecting MRS. Analyses of biomarker-behavior relationships, while exploratory, are critical to inform the interpretation and clinical plausibility of the hypothesized medication effects.

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From an alternative perspective, present results add modestly to evidence that MRS neurometabolites reflect the state of neuroinflammation in the brain. On a regional basis, the putative inflammatory markers Cho and MI[25,26,73] were lower in patients treated with a drug with known anti-inflammatory properties, while the putative anti-inflammatory marker NAA was higher, than in patients treated with placebo. These effects were accompanied by decrease in pro-inflammatory markers and increase in an anti-inflammatory marker. However, these results were not consistent between brain regions, demonstrating the complexity of attempting to understand neuroinflammatory processes within humans. This pattern may be due to differences in sensitivity between brain regions and tissue type (i.e., gray and white matter) to metabolite changes. For example, Cho is found in higher concentrations in white matter compared to gray matter[25], and the present study found changes in Cho in superior frontal white matter. Alternatively, these differences may be due to individual metabolites reflecting different aspects of inflammation that occur locally in brain regions. Within the constraints of the study, these results further motivate the use of these MRS signals in investigating suspected neuroinflammatory conditions. Of note, position emission tomography (PET) studies, ideally combined with MRS studies, will also be invaluable to confirm these relationships.

This study has several limitations. Notably, neurometabolite data were only collected at a single time-point, i.e., were cross-sectional, which precludes causal conclusions regarding ibudilast's central neuroprotective or anti-inflammatory effects. Future studies should include a pre-randomization MRS scan to evaluate the direct effect of ibudilast on neurometabolites. This study had a relatively modest sample size, particularly for the MRS component. Increased power from a larger sample is needed to replicate our findings and may reveal additional associations between neurometabolite levels and treatment outcomes. Relatedly, while this study adjusted for multiple comparisons of neurometabolites, it did not correct for the examination of three regions of interest, due to *a priori* evidence for effects of AUD in each region. Additional work should be conducted to replicate and extend the neurometabolite findings in these regions. Future studies should also employ longitudinal PET, and/or combination a PET and MRS, to causally identify ibudilast-related changes in neuroinflammation. A further limitation is the relatively short treatment time, two weeks. It is possible that longer treatment durations are needed to fully reveal the effects of ibudilast on peripheral inflammation. An ongoing 12-week clinical trial of ibudilast will provide a more complete picture of immune responses to this pharmacotherapy[74]. Furthermore, this study did not collect blood samples from healthy controls to compare peripheral markers of inflammation. Future studies should enroll healthy controls in addition to participants with an AUD to directly compare these markers and assess variation over time between groups.

In closing, this is the first study of the effect of ibudilast on peripheral and putative central inflammatory markers. Results from this preliminary study provide a potential proof-of-mechanism for ibudilast, such that it may work through a neuroprotective pathway to reduce alcohol use in individuals with AUD. Ibudilast-treated individuals had lower levels of cortical neurometabolites as compared to placebo treated individuals. The ibudilast-treated group had nominally lower levels of CRP and TNF-α/IL-10 ratio. Exploratory analyses demonstrate a predictive relationship between superior frontal white matter Cho levels and subsequent drinking in the ibudilast group, such that participants treated with ibudilast with

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low Cho levels had the fewest number of drinks per drinking day in the week following the scan. Overall, this study complements previous clinical studies of ibudilast for the treatment of AUD[42,43] by providing a potential biobehavioral mechanism for the neuroprotective effects of ibudilast. As medication development progresses, the integration of behavioral and biomarkers of medication response will be critical to advance our understanding of immune treatments for AUD and their optimal clinical application.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements:

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## Data Availability:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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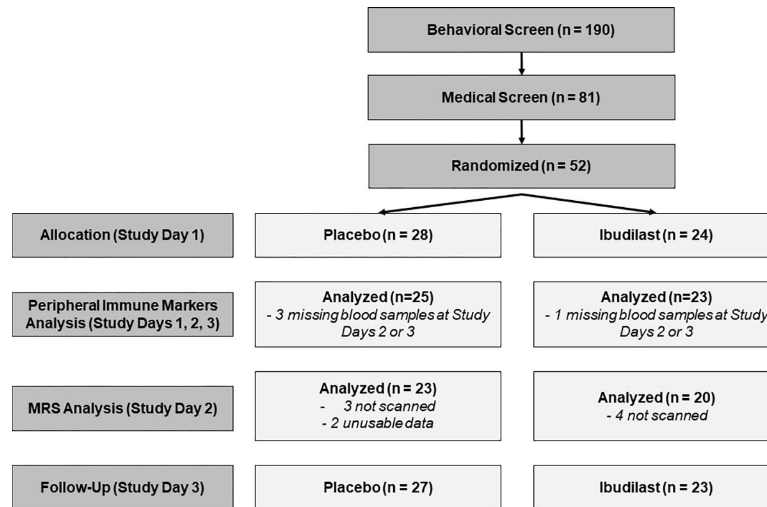


**Figure 1. Consort Diagram.**

Subject flow through the trial.

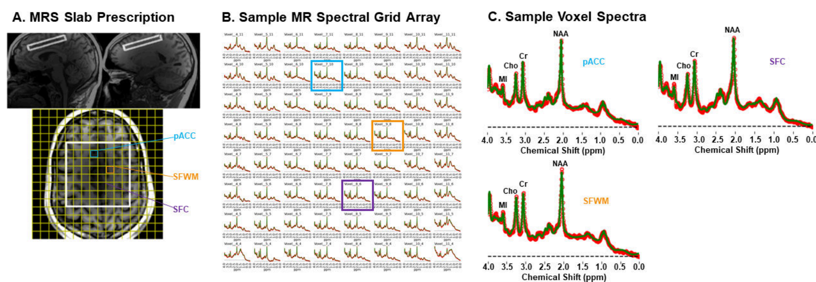


**Figure 2. MRS Acquisition Volume Prescription and Data Quality From a Representative Participant.**

**Panel A** features two sagittal (upper) and one transverse (lower) T1w MRI of the human brain showing position of the 8×8 subarray ("excitation box"; white-border) from which usable spectra are acquired within the 16×16 proton magnetic resonance spectroscopy (MRS) voxel grid (yellow). MRS was acquired with stimulated-echo acquisition mode (STEAM; TR/TE/TM=2000/20/20 ms, voxels 10×10×10 $mm^3$, 4 excitation). Sample voxels from single voxels in the pregenual anterior cingulate cortex (pACC), superior frontal white matter (SFWM), and superior frontal cortex (SFC) target volumes-of-interest (VOIs) are indicated. **Panel B** shows raw (red) and fit (green) spectra across the excitation grid with spectra from the three sample voxels magnified in **Panel C**. NAA=*N*-acetyl-compounds, Cr=creatine-compounds, Cho=choline-compounds, MI=*myo*-inositol.



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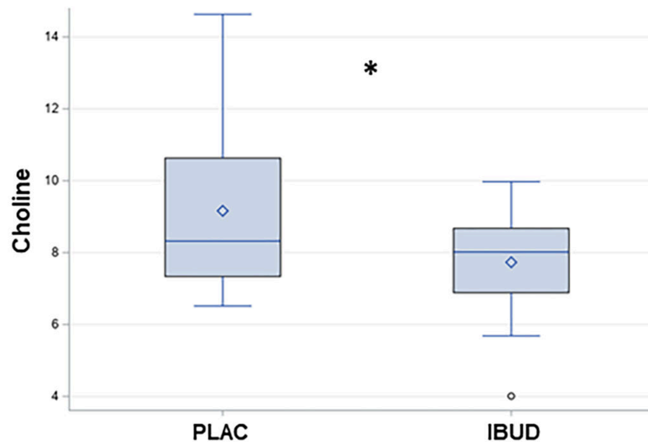


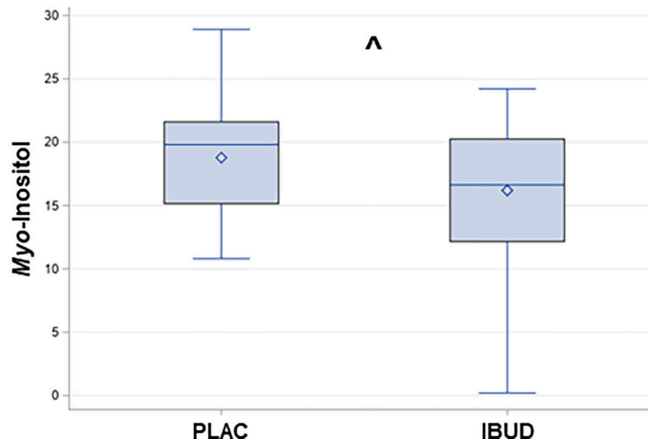


**Figure 3. Magnetic Resonance Spectroscopy Results.**

Ibudilast-treated participants had lower inflammatory neurometabolite levels relative to placebo-treated participants. In **Panel A**, participants treated with ibudilast had significantly lower choline levels in the superior frontal white matter relative to placebo-treated participants. In **Panel B**, participants treated participants had trend level lower levels of *myo*-inositol in the pregenual anterior cingulate cortex relative to placebo treated participants. * = p<0.05; ^ = p<0.08.

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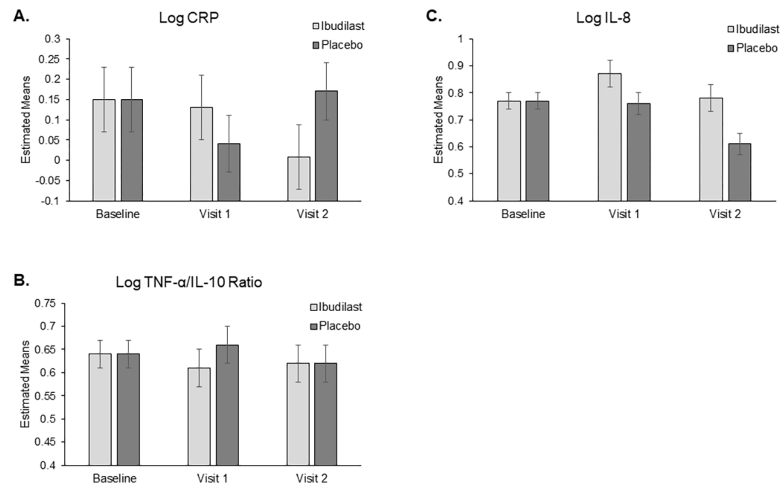


**Figure 4. Inflammatory Marker Results.**

**Panel A** shows the CRP levels over the course of the study by medication group. There was a trend-level interaction between medication and time, such that the ibudilast-treated participants had lower CRP levels at visit 2, whereas the placebo-treated participants had higher CRP levels at visit 2. **Panel B** shows the TNF-α/IL-10 ratio over the course of the study by medication group. Ibudilast-treated participants had lower ratios than placebo-treated participants across time at trend-level. **Panel C** shows the IL-8 levels over the course of the study by medication group. Participants treated placebo had lower IL-8 levels across time relative to ibudilast-treated participants. Figures are converged baselines and estimated marginal means ± SE's controlling for baseline levels, age, sex, smoking, BMI, and pre-trial drinking.

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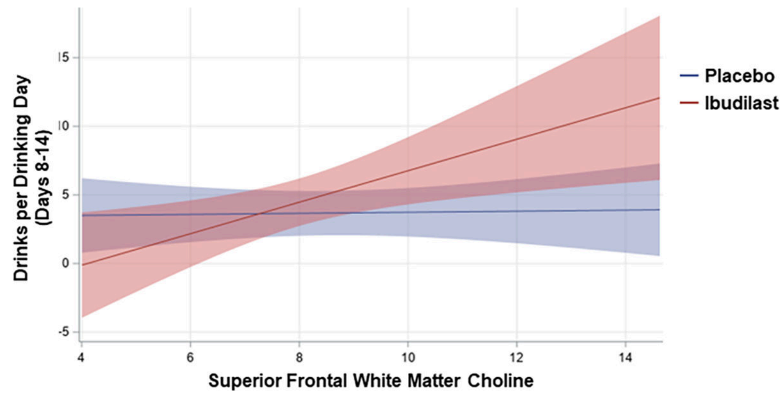


**Figure 5. Clinical Prediction.**

Superior frontal white matter choline levels at visit 1 were predictive of subsequent drinking in the ibudilast group only. Individuals who were treated with ibudilast who had low levels of choline in the superior frontal white matter had the fewest drinks per drinking day in the subsequent week.

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**Table 1.**

Demographic and Clinical Characteristics

| Characteristic<br>Mean ± Standard Deviation | Ibudilast (n=24) | Placebo (n=28) | p-value |
|---|---|---|---|
| Age (years) | 34.46 ± 9.24 | 31.07 ± 7.81 | 0.16 |
| Sex (M (%)) | 16 (66.67%) | 18 (64.29%) | 0.86 |
| Body Mass Index ($kg/m^2$) | 26.91 ± 4.72 | 26.18 ± 3.85 | 0.54 |
| Smoke cigarettes (%) | 11 (45.83%) | 14 (50%) | 0.09 |
| THC+ Urine (%) | 7 (29.17%) | 8 (28.57%) | 0.96 |
| **Pre-Randomization Alcohol Measures** | | | |
| Alcohol Withdrawal (CIWA-Ar) | 0.34 ± 1.33 | 0.37 ± 0.93 | 0.98 |
| Total Drinks (4 days) | 13.42 ± 9.68 | 12.91 ± 9.05 | 0.85 |
| Drinks per drinking day (4 days) | 5.55 ± 3.84 | 4.77 ± 2.60 | 0.39 |
| **Baseline Inflammatory Levels** | | | |
| CRP (mg/L) | 3.31 ± 3.63 | 3.45 ± 5.52 | 0.91 |
| IL-6 (pg/mL)[a,b] | 1.24 ± 1.75 | 0.60 ± 0.41 | 0.10 |
| IL-8 (pg/mL)[b] | 6.77 ± 3.86 | 7.07 ± 4.15 | 0.79 |
| IL-10 (pg/mL)[b] | 0.43 ± 0.68 | 0.21 ± 0.08 | 0.11 |
| IFN-γ (pg/mL)[b] | 6.67 ± 3.67 | 7.15 ± 4.25 | 0.67 |
| TNF-α (pg/mL)[b] | 1.05 ± 0.38 | 1.03 ± 0.29 | 0.80 |

[a]= 1 participant in the ibudilast group was missing values at baselines (n=23).

[b]= 1 participant in the placebo group was missing values at baselines (n=26).