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Treatment of Schizophrenia with l-tetrahydropalmatine (l-THP): a Novel Dopamine Antagonist with Anti-inflammatory and Antiprotozoal Activity

Abstract

Schizophrenia is a devastating and complex illness, with multiple symptom and behavioral manifestations. Antipsychotic medications are the mainstay of treatment; however, many patients only partially respond to treatment. Development of new treatment has not progressed rapidly, in part, because the underlying etiopathophysiology of the illness is not well understood. To date, all pharmacological treatments approved for use in schizophrenia involve primary modulation of the dopamine system. Many agents without dopamine action have failed to demonstrate efficacy. There is growing evidence that schizophrenia may be, in part, due to an inflammatory process and pharmacological treatment approaches that decrease inflammation have shown promise. Thus, treatments that may have anti-inflammatory properties (e.g., TNF-alpha inhibition), but also possess dopamine modulation may prove to be beneficial. This novel medication, l-tetrahydropalmatine (l-THP), has robust anti-inflammatory properties, particularly TNF-alpha and ICAM inhibition; has antiprotozoal activity; and possesses an antipsychotic-like pharmacological profile of D1, D2 and D3 receptor antagonism. The high affinity of l-THP for D1 versus D2 receptors distinguishes it from first generation antipsychotics and its D1 to D2 ratio resembles that of the superior antipsychotic, clozapine. Also, an almost identical compound, l-stepholindine (l-SPD), demonstrates robust antipsychotic activity in humans (both positive and negative symptoms) and is currently used clinically in China. l-THP has been used for over 40 years clinically in China, has a good safety profile to date, and represents a novel and exciting mechanism for schizophrenia treatment. Initial safety data from our phase I study of l-THP (20 healthy controls) shows excellent tolerability and lack of any substantial side effects. L-THP has been tested in outpatient drug abuse trials for 4 weeks with good safety data, (Hu et al 2006, Yang et al 2003). Yang et al (2003) randomized this medication in over 120 participants for 4 weeks with 4 week observation without any notable side effects.

We will test this compound (30 mg BID) as an adjunct treatment in a randomized, double-blind, 4-week trial, in which we will assess treatment efficacy, changes in peripheral cytokine concentrations, and, secondarily, antiprotozoal effects, (antibody titers to *Toxoplasma gondii),* an infection that is known to occur at higher rates in schizophrenia than healthy controls and may be related in part to the illness.. We hypothesize that adjunctive l-THP added to antipsychotics in people with schizophrenia will significantly improve positive and negative symptoms, cognition, and inflammation, particularly through TNF-alpha inhibition, and may have antiprotozoal effects. We hypothesize it will be effective and safely tolerated. In addition subjects can optionally participate with the addition of magnetic resonance imaging (MRI).

Research Strategy:

We have recently been funded by the Stanley Medical Research Institute to investigate l-THP, a novel drug having anti-inflammatory and antiprotozoal activity, as well dopamine antagonism and modulatory activity at GABA(A) and 5-HT 1A and 2A receptors. This drug could represent a breakthrough treatment in people with schizophrenia.

Study Aims:

1. To determine if adjunctive l-THP is superior to placebo for the treatment of positive and negative symptoms of schizophrenia. (PRIMARY OUTCOME)

2. To determine if adjunctive l-THP is superior to placebo for reducing inflammatory response, assessed through peripheral cytokine concentrations (serum TNF-alpha, IL-6 and ICAM-1 (primary); IL-1beta IL2, IL8, IL10, IL12, IL17, and IFNgamma) (secondary)), in people with schizophrenia

3. To determine if adjunctive l-THP is superior to placebo for the treatment of cognitive impairments and depressive symptoms in people with schizophrenia

4. To examine the tolerability and safety profile in l-THP relative to placebo in people with schizophrenia

5. To evaluate the effects of l-THP in a subgroup of people who are positive for antibodies to *Toxoplasma gondii* at baseline (Exploratory Aim)

Optional MRI Aims:

1) To determine the effect of adjunct l-THP on brain glutamate and GABA concentrations and blood flow as measured with proton magnetic resonance spectroscopy (1H-MRS) and arterial spin labeling (ASL) respectively.

We hypothesize that the addition of l-THP will significantly change glutamate, GABA and blood flow concentrations from pre- to post-l-THP treatment.

2) To determine if the change in glutamate, GABA and blood flow relates to the improvement in positive, negative, and cognitive symptoms.

We hypothesize that the amount of glutamate, GABA and blood flow change will correlate with the magnitude of improvement in positive, negative, and cognitive symptoms.

3) To determine if the change in glutamate, GABA and blood flow relates to the change in peripheral inflammatory markers.

We hypothesize that the amount of glutamate, GABA and blood flow change will correlate with the reduction in serum proinflammatory cytokines.

We plan to conduct a double-blind, randomized, parallel group clinical trial of adjunct l-THP vs. placebo in 85 people with schizophrenia who are maintained on a stable antipsychotic regimen. This study will consist of 4 weeks of randomized treatment.

Schizophrenia and Inflammation: Schizophrenia is a devastating and complex illness, with multiple symptom and behavioral manifestations. Antipsychotic medications are the mainstay of treatment; however, many people with schizophrenia only partially respond to treatment. Treatment development has not progressed rapidly, in part, because the underlying etiopathophysiology of the illness is not well understood. To date, all pharmacologic treatments approved for use in schizophrenia involve modulation of the dopamine system. Many agents without dopamine involvement have failed. There is growing evidence that schizophrenia may be, in part, due to an inflammatory process (Meyer et al 2011, Debnath and Venkatasubramanian 2013) and pharmacologic treatment approaches that decrease inflammation show promise (Sommer et al 2012, Keller et al 2013). Thus, treatments that encompass both effects on inflammation and also antagonism at dopamine receptors may prove of benefit in people with schizophrenia. TNF-alpha is an inflammatory mediator that has been implicated extensively in schizophrenia. A recent meta analysis found TNF-alpha to be a significant trait marker that is elevated consistently in schizophrenia (Miller et al., 2010). Levels of TNF receptor 1 (TNFR1) mRNA are increased post-mortem in several brain regions of people with schizophrenia, demonstrating not only peripheral inflammation but also brain involvement of TNF-alpha, which has not been shown in other psychiatric disorders (Dean et

al 2013). TNF-alpha might contribute to the pathogenesis of schizophrenia by activation of the hypothalamo-pituitary-adrenocortical (HPA) axis, activation of neuronal serotonin transporters, stimulation of indoleamine 2,3-dioxygenase, which increases kynurenic acid, by immunologically mediated destruction of neurons, or release of glutamate (Himmerich et al 2009). Interleukin-6 (IL-6) and intracellular adhesion molecule (ICAM)-1 are both mediated by TNF-alpha. (Yu et al 2008). IL-6 is consistently shown to be elevated in schizophrenia (Zakharyan et al 2012), while ICAM-1 levels are significantly lower in schizophrenia compared to healthy controls. Some genetic polymorphisms in ICAM have been related to schizophrenia (Kronig et al 2005).

Effects of l-tetrahydropalmatine (l-THP) on inflammation: Tetrahydroprotoberberines (THPBs) are a class of alkaloids isolated from the Chinese species *Stephania intermedia* of the Cordydalis ambigua herbs. THPBs have effects on inflammation; the unpurified herb has been widely used in China since the 1970s for inflammatory disorders such as endometriosis, arthritis, menstrual inflammation, and pain (Zhao et al 2011). L-tetrahydropalmatine (l-THP) is one of the most interesting THPBs, used in China under the trade name Rotundine. l-THP has robust anti-inflammatory properties in immune pathways (Zhang et al 2005) implicated in schizophrenia. For example, l-THP decreases TNF-alpha synthesis and neutrophil infiltration, thereby lessening the extent of apoptosis through a decrease in nitric oxide (NO) synthesis (Han et al 2012). It has neuroprotective effects, possibly modulated through neuroinflammation, and decreases glutamate concentrations and reduces cerebral ischemia in mice (Zhang et al 2004). These same effects protect against myocardial ischaemia-reperfusion injury in rats (Han et al 2012) by suppression of adhesion molecules and cytokine release. l-THP markedly attenuated adhesion molecule expression induced by LPS in human umbilical vein endothelium cells (HUVEC). ICAM-1 and E-selectin expression were both markedly attenuated, suggesting that l-THP represents a promising candidate for the treatment of inflammation (Zhang et al 2005).

Effects of l-THP on dopamine: In addition to its anti-inflammatory properties, l-THP has a pharmacologic profile similar to many medications that are effective for schizophrenia. Among the class of THPBs, l-THP and l-stepholidine (l-SPD) are two of the most potent binders to dopamine receptors (Xu et al 1989). l-THP has antagonist properties at D1 and D2 receptors. The higher affinity of l-THP for D1 (Ki=124 nM) versus D2 (Ki=388 nM) receptors resembles that of clozapine (Wang and Mantsch 2012), distinguishes it from conventional antipsychotics. l-THP also has antagonistic properties at D3 receptors and may exert antagonism at presynaptic autoreceptors, leading to increased dopamine release in the striatum. In rats, l-THP acts on nigro-striatal neurons as a dopaminergic antagonist that can block both pre- and postsynaptic receptors. l-THP functions as an alpha-1 adrenergic receptor antagonist, a positive allosteric modulator of GABA(A), and modulates alpha -2 adrenergic receptors and 5-HT 1A receptors (Lu et al 1996, Halbsguth et al 2003). L-THP also has serotonin (5HT 1A and 2A) agonist activity (Qian et al 2012).

l l-SPD has a very similar pharmacologic profile to that of l-THP. In animal models, it increases the hind-limb retraction tie on the paw test and reverses apomorphine-induced disruption in prepulse inhibition. Overall, L-SPD has effects similar to clozapine but different than haloperidol (Ellenbroek et al 2006). For example, it preferentially increases Fos expression in corticolimbic areas, like clozapine and unlike haloperidol, possibly through non-DA mechanisms (Mo et al 2005). Three clinical trials examined the effects of l-SPD in

schizophrenia. In the first placebo-controlled study, l-SPD (150 mg daily) for 8 weeks, together with a first generation antipsychotic, significantly improved BPRS scores and reduced tardive dyskinesia relative to placebo (Cai et al 1988). In the second study, l-SPD monotherapy (400-600 mg daily) (N=13) significantly improved global symptomatology--baseline and endpoint Global Assessment Scale (GAS) scores were 39.4 ± 7.5 and 60.6 ± 12.4, respectively ($p<0.05$) (Wang et al 2000). The most recent study (Wu et al 2003) compared the effects of l-SPD 225-625 mg daily (N=31) to perphenazine 16-44 mg daily (N=30) in an 8-week double blind randomized trial. l-SPD had earlier onset of effect and was significantly more effective for positive and negative symptoms than perphenazine and had no extrapyramidal side effects. The dopamine and 5HT1A effects of the THBPs suggest that they are promising novel lead drugs for the treatment of schizophrenia (Sun et al 2013).

Effects of l-THP on Protozoal Infections: Accumulating evidence shows that antibodies to *Toxoplasma gondii (T. gondii*), a protozoa that infects humans, along with its tissue cysts, are present in people with schizophrenia at increased rates and may be associated with neurologic and psychiatric manifestations of schizophrenia. At least 38 studies found *T.gondii* to be present in people with schizophrenia more frequently than in controls, with an odds ratio (2.71-2.73 95% CI) (Torrey et al 2012) suggesting *T gond*ii is a moderate risk factor for schizophrenia. l-THP has strong to moderate anti-protozoal activity (IC50 0.08 ± 0.001 – 2.4 ± 0.001 ug/ml, SI 1.5-1,154) (Malebo et al 2013, Baghdikian et al 2013). While this may not be its primary mechanism of efficacy in schizophrenia, this anti-protozoal effect could contribute to improvements in symptoms in those positive for *T.gondii,* so we will perform exploratory analyses in this subgroup.

Research Design and Outcome Measurements:
We propose to conduct a double-blind, randomized clinical trial of four weeks of treatment with adjunct l-THP vs. placebo in 85 people with schizophrenia who are maintained on a stable antipsychotic regimen (inpatients and outpatients). Participants will be seen weekly and given their medication on a weekly basis. The Schedule of Events is listed below. In addition to the PI, primary research staff will include a medically accountable physician, a regulatory compliance specialist, a study coordinator, a research nurse, a study pharmacist, a data analysist, a statistician, a research study supervisor and several co-investigators and staff. Participants will receive state-of-the-art care at the Maryland Psychiatric Research Center and 24-hour availability of research personnel.

Medical Workup: At baseline, all participants will undergo a medical work-up, including a comprehensive medical history and physical examination. We will obtain baseline, midpoint and end-of-study EKG, CBC, Chemistry Panel, liver enzymes, thyroid panel and urinalysis assessments. Weight, height and vital signs (heart rate, pulse, blood pressure) will be assessed weekly throughout the trial. The diagnosis of schizophrenia from the medical record will be confirmed by a structured psychiatric examination, the Structured Clinical Interview for DSM-IV (SCID). Following assessments of recent symptom history and current symptom levels, participants will be reassessed one week later, prior to randomization, to verify their continued symptom stability. Lab values that are considered to be abnormal may be repeated to verify accuracy.

Inclusion Criteria:

1) DSM-IV diagnosis of schizophrenia or schizoaffective disorder;
2) minimum score of 45 on the total Brief Psychiatric Rating scale; or a CGI socre of 4
3) Age 18-64 years;
4) currently taking antipsychotic regimen with no dose changes in last 30 days; and
5) ability to consent determined by a score of 10 or greater on the Evaluation to Sign Consent.

Exclusion criteria:
1) women who are pregnant, nursing, or not using effective contraception (if capable of getting pregnant);
2) current organic brain disorder or mental retardation;
3) medical condition whose pathology or treatment could alter the presentation or treatment of schizophrenia or significantly increase the risk associated with study medication. This includes HIV, kidney disease, congestive heart failure, pheochromocytoma, untreated hyperthyroidism, dehydration, fever, uncorrected congenital heart defect, seizures, electrolyte imbalance, uncontrolled diabetes mellitus, porphyria variegate, superventricular tachycardia, atrial fibrillation, cardiomyopathy, or cancer. This also may include other medical conditions where the medically accountable investigator in the study does not think it would be in the best interest of the participant to participate in the study.
4) current (past month) substance abuse or dependence (DSM-IV criteria) other than nicotine or caffeine; substance use, per se, will not be exclusionary;
5) inability to provide valid informed consent.
6) inability to understand English
7) inability to cooperate with study procedures
8) taking herbal or homeopathic medications where the metabolism of the drug is not known
9) FOR MRI ONLY Contraindication for MRI scanning (e.g. metal in body, pacemaker).

Symptom Outcomes: The primary symptom outcomes for this trial will be psychosis and negative symptoms.

The *Brief Psychiatric Rating Scale (BPRS)* (Overall and Gorham 1962) will be administered at baseline and at the end of each week. The BPRS is a widely used symptom rating scale in psychiatric research, is highly sensitive to change, and has excellent inter-rater reliability with appropriate training of raters. At MPRC, raters are required to show agreement >0.8 with gold standard ratings of training tapes.. Psychosis (sum of BPRS items for conceptual disorganization, hallucinations, delusions and suspiciousness) is one of two primary symptom outcomes. BPRS total score is a secondary symptom outcome.

The Schedule for Assessment of Negative Symptoms (SANS) will be used to assess negative symptoms (Andreasen 1983). In this trial, we will use an adaptation of the SANS developed by the CONSIST investigators which allows for improved assessment of avolition in inpatient studies. In recent clinical trials at the MPRC, the SANS has shown high (>0.8) within-patient intra-class correlations (ICC). Inter-rater training and achieved reliability at the MPRC on the SANS is comparable to that for the BPRS. The SANS total score will be the other primary symptom outcome.

Calgary Depression Rating Scale (CDS) (9 items) is designed specifically to assess depressive symptoms in people with schizophrenia and has been shown to be a reliable measure with good construct validity (Addington 1993). The CDS total score and suicide item will be secondary symptom outcomes.

The Columbia-Suicide Severity Rating Scale (C-SSRS) (Posner et al 2011) assesses suicidal ideation/behavior.

Clinical Global Impression Scale (CGI): the CGI severity of illness and change scores items will assess severity of illness and global changes**.**

Brief Negative Symptom Scale (BNSS) (Strauss et al 2012) will also be used as a measure of negative symptoms. It is a brief 13 item scale with good psychometric properties that provide good domains of negative symptomatology measurement.

Peripheral Cytokines and Laboratory Measures (Baseline and endpoint during the trial): Plasma TNF-alpha, IL-6, and ICAM-1 (primary) and other inflammatory cytokines (IL-1beta IL2, IL8, IL10, IL12, IL17, highly sensitive c reactive protein, and IFNgamma) (secondary) will be measured in peripheral venous blood at baseline, 2 weeks, and at the end of the study. Serum cytokines will be measured by the Cytokine Core Laboratory at the University of Maryland using Luminex® 100 Multianalyte System or ELISA. Plasma levels of l-THP and its metabolites will be measured at midpoint and endpoint at the University of Maryland School of Pharmacy Pharmacokinetics Laboratory. CBC, Chemistry panel, EKG, liver enzymes, and thyroid function will be done at baseline, midpoint, and endpoint of the study.

Antibodies to *T. gondii* will be measured at baseline and endpoint by solid phase immunoassay by Dr. Robert Yolken at Johns Hopkins University. The results are expressed in terms of ratios to arbitrary standards and are thus useful for comparison within groups. In addition to *T. gondii*, the Hopkins laboratory will measure a panel of viruses, including Herpes Simplex Virus Type 2 (HSV-2), Cytomegalovirus (CMV), Epstein Barr Virus (EBV-EBNA), Varicella Zoster Virus (VZV), Human, Herpes Virus Type 6 (HHV-6), Influenza A Virus (H3N2), Influenza B Virus and Gliadin IgG. Values greater than 1 are considered positive for all titers except for HHV6, which is considered positive if greater than 6. *T. gondii* serotyping for type 1, type 2, and type 3 will also be performed.

Measures of Neurocognition (Baseline and endpoint). We will use the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) Consensus Cognitive Battery (MCCB), which is the standard for clinical trials in schizophrenia. The tests include Trail Making Test, Part A; BACS symbol coding subtest; Hopkins Verbal Learning Test, revised; immediate recall with three learning trials only; Wechsler Memory Scale, spatial span subtest; Letter-Number Span test; Neuropsychological Assessment Battery, mazes subtest; Brief Visuospatial Memory Test, Revised; Category fluency test, animal naming; Mayer-Salovey-Caruso Emotional intelligence Test, managing emotions branch; and Continuous Performance Test, Identical Pairs version. These tests have good reliability and validity and require about 90 minutes. In recent multicenter clinical trials in which the MPRC has participated, individual tests of the MCCB have had ICCs between 0.6 and 0.8, and the overall MCCB composite score had an ICC of 0.9.

Measures for Safety and Tolerability: We will do the following tests weekly throughout the study:

Simpson Angus Extrapyramidal Symptom Rating Scale (SAS) (Simpson and Angus 1970) (a modified 11-item version of the SAS will be used to assess extrapyramidal symptoms (EPS)),

*Barnes Akathisia Scale (BAS) (*Barnes 1989) (a 4-item scale designed to assess objective and subjective components of akathisia)

Side Effect Checklist (SEC) is designed to assess commonly occurring side effects and those relevant to I-THP

Epworth Sleepiness Scale (ESS) is an 8-item scale rated 0-3 that evaluates daytime sleepiness

Optional Magnetic Resonance Imaging (MRI).

Magnetic resonance techniques allow us to non-invasively examine the human body in vivo and gather information about different structures, functions, connectivity, and chemistry. Brain anatomy, chemistry, and blood flow will be investigated with noninvasive magnetic resonance (MR) techniques. MRI scanning will take place at the UM Center for Brain Imaging Research. MRI scans will be conducted on a Siemens 3 Tesla magnet).

Subjects will be screened twice for eligibility to enter the MR scanner, once by the research assistant and again by the MR technologist immediately prior to entering the MR suite. Prior to entering the MR suite, subjects will be asked to remove all metal from their clothing and/or change into MRI-compatible clothing before undergoing a study protocol. To protect the subject's hearing, earplugs or earphones will be provided, but the subject will still be able to hear instructions. Subjects will lay down on the examination table, be made as comfortable as possible, and moved inside the MR scanner for the duration of the study. Two anatomical scans will be conducted, T1-weighted imaging and diffusion tensor imaging (white matter anatomical imaging). Glutamate concentrations will be collected with proton magnetic resonance spectroscopy (1H-MRS) and blood flow will be collected with arterial spin labeling (ASL). Functional connectivity will be examined with resting-state functional MRI (fMRI). The total MR scan time will be about 1.5 hours. No intravenous contrast will be injected.

Subjects will be communicated with by the MR technician and study team members in the MR control room throughout the scan and reminded that individual(s) in the control room can see and hear them at all times. Each subject will be reminded that if they become uncomfortable and wish to leave the magnet, they can tell the study staff or MR technologist and they will be promptly removed from the scanner. Once the study protocol is completed, the subject will be removed from the MR scanner.

Schedule of Events						
Encounter # →	1 (Screening)	2 (Baseline)	3	4	5	6 (Endpoint)
Week of Study →	W -1-4	W0	W1	W2	W3	W4
Procedure ↓						
Informed consent and ESC	X					
Demographic Forms	X					

Past Psychiatric and Medication History Form	X					
SCID-Interview	X					
Medical History	X					
Physical Exam and height	X					
SMAC, BUN, glucose,	X	X		X		X
CBC, UA, lipids, c-reactive protein		X		X		X
LFT		X	X	X	X	X
Urine Pregnancy Test (female participants)	X	X	X	X	X	X
EKG	X	X		X		X
Fagerstrom Test for Nicotine Dependence		X				X
QSU-B (Questionnaire for Smoking Urges - Brief) and the Nicotine Dependence 15 item		X				X
MATRICS Consensus Cognitive Battery		X				X
Clinical Global Impression Scale (CGI)	X	X				X
Brief Negative Symptom Scale (BNSS)		X				X
Brief Psychiatric Rating Scale	X	X	X	X	X	X
Positive and Negative Syndrome Scale (PANSS)		X				X
Scale for the Assessment of Negative Symptoms		X	X	X	X	X
Calgary Depression Scale		X	X	X	X	X
C-SSRS		X	X	X	X	X

Simpson Angus Scale		X				X
Barnes Akathisia Scale		X				X
Cytokines		X				X
T. gondii, HSV		X				X
l-THP and metabolite levels			X	X	X	X
Prolactin Level		X				X
Side Effect Checklist and ESS		X	X	X	X	X
Vital Signs, including weight, BP and pulse	X	X	X	X	X	X
Pill Count			X	X	X	X
Optional MRI		X				X

Treatment and Acquisition: Treatments will be assigned at random, using separate permuted block randomization sequences. The l-THP (or matching placebo) will be dosed as one capsule (30 mg) twice daily (total 60 mg daily). The l-THP has already been prepared at the University of Maryland School of Pharmacy to Chemistry, Manufacturing and Controls CMC standards after obtaining the purified l-THP from China and receiving the IND from the FDA. Identical placebo and active capsules will be manufactured and sent to the Maryland Psychiatric Research Center Pharmacy, where they will be stored and dispensed.

Preliminary Data on Safety: In conjunction with Dr. Jia Bei Wang from the University of Maryland School of Pharmacy, l-THP has been formulated in a GMP laboratory as 30 mg tablets, which we plan to give twice daily, the dose found effective in prior studies. Since February, 2013, our group has enrolled 20 healthy participants into a 3-day inpatient study to test tolerability and safety of l-THP. These controls (without drug use histories) experienced no significant or unexpected side effects (a 25-item list of potential side effects was read to participants daily) and no clinically significant laboratory changes were seen.

We have been approvd by the FDA with a new IND. l-THP is widely used in China for over 40 years and approved by the Chinese SFDA (FDA equivalent) (Chu and others 2008; Jin 1987). The drug, according to the Chinese pharmacopeia and drug labels, is safe at the recommended therapeutic dosage range (60-120mg). Adverse effects include drowsiness (77% at dose above 90mg), dizziness, and nausea (rare). Although l-THP is a main active ingredient from a Chinese herb, it is regulated by the Chinese SFDA, and successfully completed a medication development process in the early 1960s. Therefore, it should not be considered an unregulated herbal product. Overdose has caused respiratory inhibition and extrapyramidal symptoms. Literature search (in English and Chinese) found a few reported cases of allergic reaction and CNS reactions after use of l-THP, but no reports of liver, kidney, or cardiovascular toxicity associated with the use of l-THP in humans. Our phase I study also found no untoward side effects. In addition, l-THP has been safely used in outpatient studies for heroin use (Hu et al 2006, Yang et al 2003). The 60 mg per day dose, based on previous studies, should minimize sedation.There are no known and reported drug interactions in humans however to be very cautious based on a paper with l-THP was reported to have some

inhibition in vitro of the CYP450 2D6 pathway (Sun et al 2013) we have created an appendix of mediations that are primarily metabolized by the 2D6. We initially started participants in the inpatient setting and saw patients more frequently than once weekly. The study is enrolling outpatients that are seen once weekly. We do not believe the risk of CYP 2D6 inhibition is high and were cautious when beginning the study in the first few participants. We will not include this as exclusionary but if participants are on these medications they will be evaluated weekly for possible drug interactions.

Recruitment: Recruitment for this study will occur through 3 primary sources. 1) We will be specifically recruiting in the inpatient and outpatient clinics at the Maryland Psychiatric Research Center, the primary clinics used for study recruitment at MPRC. 2) Our program in the TRP, through funding from the National Institute of Drug Abuse (NIDA), has built a large screening database over the past 3 years with over 200 people recruited by local advertisements. 3) We will also utilize a developed recruitment network with community mental health centers, the Practice Research Network (PRN). This PRN was developed in 2008-2009 (funded by IP-RISP, PI Dr Buchanan) to provide a network for schizophrenia research in the State of Maryland. We are confident we will have no trouble recruiting participants. The PI has successfully completed all previously funded studies. As an example, Dr. Kelly just recently completed an NIMH R21 study of minocycline adjunct to clozapine in schizophrenia. Recruitment began in June of 2011 and all 50 participants were enrolled in two years, the participant last finishing within 2.25 years of the start of recruitment.

Participant Payment: Participants will receive $40 for screening visits and $40 for all 4 weeks in the study, and $25 for each completed MRI. This totals to a payment of $200 for study completion, without the MRI scans and $250 with the MRI scans. Transportation will be provided by prepaid taxi rides. Meals may be provided if the study visit falls near meal times. If an additional screening visit is required participants will be paid $40 more for the extra visit.

Management of Data: Data collected for this study will be entered into the MPRC research data base using the usual procedures developed for MPRC clinical trials. This study will be reviewed UMB IRB and by a Data Safety Monitoring Board (DSMB).

Data Analysis: As specified above, our primary symptom outcomes will be the BPRS psychosis score and SANS total score, assessed at baseline, midpoint, and end of study. We will analyze these two primary outcomes (as well as secondary outcomes collected on the same schedule) in intent-to-treat analyses using mixed models for unbalanced repeated measures (MMRM) analysis of covariance (ANCOVA), including all available observations from participants who complete at least one follow-up rating , where each primary outcome measure is adjusted for its baseline score. Use of mixed models has the advantages that data from participants who drop out early can be included in the analysis and that estimates of treatment effects will be unbiased by dropout, if reasons for dropout ignorable with respect to the outcome, given the observed data. These models will have the basic form: follow-up score = baseline score + week + treatment + treatment x week, where week is a set of categorical indicators defining which week the follow-up score comes from.

The primary test for treatment effect will be a post hoc contrast from the MMRM outlined above, comparing the mean outcomes scores at week 4, adjusted for baseline, in patients assigned to l-THP or placebo. We have chosen to focus on week 4 differences because of uncertainty about when treatment differences will emerge in the two strata. To take account of

having two primary outcomes, in testing for differences we will use the Benjamini-Hochberg False Discovery Rate (FDR) method. If the p-values for both primary outcomes are <0.05, both null hypothesis will be rejected. Otherwise, if one p-value is <0.025, the corresponding null hypothesis will be rejected. Secondary outcome measures collected at baseline and end of study will be analyzed as follows, testing at unadjusted α=0.05 for secondary analyses. Change scores for cytokines, which often have highly skewed distributions, will be analyzed using the Wilcoxon rank sum test. The FDR method will be used to adjust for multiple comparisons for the three primary cytokines (serum TNF-alpha, IL-6 and ICAM-1). Four weeks is sufficient time to detect an antipsychotic effect. Recently shorter clinical trials of 2 to 4 weeks duration have been recommended as a viable option for early identification of drug efficacy. Several large studies using a wide range of antipsychotics have found the power analyses show that placebo-drug differences are robust by 2 weeks (Kinon et al 2010a, Kinon et al 2010b, Giegline et al 2012). Thus, most studies show most benefit within the first 4 weeks of treatment and response at 2 weeks predicts response at 6 weeks as does nonresponse at 2 weeks predicting nonresponse at 6 weeks (Kinon et al 2010a)

The MATRICS battery composite score, a normed average of the T scores representing different cognitive domains, will be our primary neurocognitive outcome measure and will be analyzed using analysis of covariance, adjusting for baseline MCCB composite score. Daytime drowsiness scores (from ESS) at baseline will be compared between treatment groups using analysis of covariance, adjusting for ESS scores measured at baseline.

T.gondii antibodies we conservatively estimate to occur in 50% of people with schizophrenia (Hamidinejat et al 2010). In an exploratory analyses in participants initially seropositive, we will compare the two treatment groups on the proportion of participants who become seronegative at the end of the trial, and will compare symptom changes from baseline to week 4 in those remaining seropositive versus converting to seronegative. Dr. Robert Yolken will advise and oversee the research plan, data collection and analysis of this through the Stanley Division of Developmental Neurovirology at Johns Hopkins University. We are unsure if we will see significant changes in antibody titers, but this preliminary aim will allow us to see if antiprotozoal effects leads to improved titers. We will also be able to test if those with antibodies show more improvement in symptoms than those without.

MRI: Glutamate and GABA concentrations and blood flow will be the primary outcome variables. Comparisons between the pre and post l-THP treatment will be conducted with t-tests (MRS) and MANOVA (multiple brain regions for blood volume). Specific brain regions that will be investigated include the anterior cingulate, hippocampus, and dorsolateral prefrontal cortex.
To test specific aims 2 and 3, linear regression model will be used to estimate if glutamate and GABA concentrations and blood flow is correlated with positive, negative, cognitive, and peripheral measures of inflammation.

Power: With n=85 participants, and 15-20% attrition by end of study, consistent with recent adjunctive treatment studies at MPRC, we anticipate having end-of-study ratings on at least 52 participants, approximately evenly divided between placebo and l-THP. We estimate approximate power for the primary outcome using the main effect of treatment in a simple ANCOVA, adjusting for baseline. The effect size E, detectable by a repeated measures ANCOVA for a given sample size, n, per group is given by: $E^2 = 2(z_{1-\alpha/2} + z_{1-\beta})^2 (1-\rho^2)/n$, where $z_{1-\alpha/2}$ and $z_{1-\beta}$ are the quantiles of the normal distribution corresponding to the specified Type I

error rate and power, respectively. In this formula, the term $(1-\rho^2)$ reflects the reduction in the variance in follow-up scores from adjusting for the baseline score. To estimate power conservatively, we set $\alpha=0.025$, two-sided, to reflect use of the FDR method. We anticipate being able to detect an effect size of $d=0.57$ if $\rho=0.7$ or $d= 0.48$ for $\rho =0.8$, consistent with ICCs we have seen in trials of similar length in clinically stable participants for the BPRS psychosis score and SANS. For the MCCB composite score, we and others have found $\rho>0.9$. Testing at $\alpha=0.05$, two-sided, we should have power=0.80 to detect an effect size $d=0.349$ on the MCCB composite.

HUMAN SUBJECTS

1. Protection of Human Subjects

a) Human Participant Involvement, Characteristics and Design

The proposed study meets the definition of a "Clinical Trial" and it will be registered in ClinicalTrials.gov. Participants involved in this study will be diagnosed with schizophrenia or schizoaffective disorder and will be stabilized on current antipsychotic medications. Included participants must also be judged competent to consent by the Evaluation to Sign Consent (ESC) questionnaire, and provide voluntary informed consent. Women with reproductive potential must agree to the use of medically approved birth control, which includes oral contraceptives, condoms, diaphragms, and intrauterine device. Pregnant and nursing women will be excluded.

Project Timeline:

Year	Start-Up Activities	Recruitment and Study Activities	End of Study Activities
Year 1	- Finalize protocols, IND amendment, data base design, inter-rater reliability - IRB, and clinical trials.gov registration	- Enroll approximately 18 participants -Ongoing rater reliability	
Years 2-4		- Enroll approximately 25 participants -Ongoing rater reliability -Annual Reports	
Year 5		- Enroll approximately 17 participants -Between site monthly meetings	- Conduct data cleaning and analyses and manuscript preparation. -Presentation of results at annual meetings -End of Study Reports

b) Sources of Materials

Material sources for this study include computerized and hard copy research records and blood and urine specimens. All research records will be kept in locked files with participants'

evaluation study materials identified by code only. A separate file will hold the code key. Participants will not be personally identified in any publications or reports of the study. Any data used will be re-copied to research files with the participant identified by code only. The highest standards of participant confidentiality will be kept, and no participants will have identifiable information available to anyone other than authorized study staff. Computerized records of data are kept in a password-only accessible computer in a locked room. Appropriate firewalls and protections of computerized data are maintained to ensure that entry by those other than research personnel is not possible.
Data will be entered into a Microsoft Access database. All forms are identified within this database by a unique participant ID number, participant initials, and the protocol ID number and acronym, together with the protocol phase and visit. The protocol data collection schedule is kept in a data base table and is used to monitor the progress of participants through the protocol, missing assessments, and other protocol deviations during the study. Data entry screens incorporate range checks or lists of valid responses for each item. Forms with missing or invalid data in key identifying fields are referred back to raters for correction before entry. Participant confidentiality is maintained by restricting study data access to specified study personnel. Most authorized personnel will have read-only access, and write/edit access will be restricted to data entry and data management staff assigned to the study. An electronic audit trail records all changes to the data base once data have been entered. The database resides on a central server, and all server data are backed up several times weekly. Access to the server from outside the MPRC is restricted by a firewall. Norton Anti-Virus software, updated automatically whenever new virus data files are provided, is installed on the server, and all computers are linked to the server. Development of the data management system for this study will be facilitated by the existence of this data base structure, which already contains tables and data entry screens for many of the assessments used in this study.

All of the above data will be gathered specifically for research purposes only. Information collected in this study will be stored and managed by the Biostatistics and Study Management Core at MPRC. Access to research data stored in the database will be limited to investigators who have been granted user names and passwords by the PI.

All blood and urine specimens are for research purposes only and will be identified at the laboratory by code only. Conventional laboratory tests will be performed and evaluated by Lab Corp® using GLP procedures and policies. Blood for the immune measures will be kept in a secure -80ºC freezer until ready for analysis.

c. Potential Risks to the Participants

The major risks of the study are the risk for side effects from l-THP. L-THP has more than 40 years of safe clinical use in China, and its toxicity profile in a variety of patient groups is well established (Chu and others 2008; Jin 1987).According to the Chinese pharmacopeia and drug labels, l-THP is safe at the recommended therapeutic dosage range (60-120mg). Adverse effects include drowsiness (77% at dose above 90mg), dizziness, nausea (rare), and neutropenia. Although l-THP is a main active ingredient from a Chinese herb, it is regulated by the Chinese SFDA and successfully completed a medication development process in the early 1960s. Therefore, it should ***not*** be considered an unregulated herbal product. Overdose has caused respiratory inhibition and extrapyramidal symptoms. Literature search (in English and Chinese) found a few reported cases of allergic reaction and CNS reactions after use of l-THP, but no reports of liver, kidney, or cardiovascular toxicity associated with the use of l-THP in humans. Our preclinical and phase I data submitted to the FDA did not show any unexpected or

serious side effects. Existing data does not suggest weight gain or metabolic effects; however, given that these are side-effects from many standard antipsychotics, we will carefully monitor for them.

Other risks include bruising from peripheral venous blood collection, skin irritation from EKG electrodes, and boredom from cognitive testing. The risk from research interviews is minimal and relatively uncommon. During assessments, participants may be uncomfortable discussing their smoking habits or mental health history and treatments. Participants may become frustrated and tense when they encounter difficulty completing tests or interviews. Careful planning and observation of the participant's response to these sessions will allow the testing to be completed with a minimum of discomfort. Participants may take breaks at any time to alleviate discomfort. All interviewers are trained to recognize signs of distress or anxiety. The participant will be reminded that they can refuse to answer any question that makes them uncomfortable and may take breaks whenever they are needed. There is a slight risk of breach of confidentiality. All data will be coded with an ID number that is unique. All data, including information from chart reviews, therapist reports, and laboratory results, will be identified by ID number only. Only the study team will have access to the link between the ID number and participant's name. Data containing names and personal information will never be included in published materials.

MRI: The effects of magnetic fields in an MR scanner have been extensively studied, and the risks with an MR exam are low, particularly if the patient qualifies for the testing based upon the screening questions. Both the magnetic field strength and the superconductor represent potential hazards: ferromagnetic objects introduced into the field can become high velocity projectiles capable of causing serious injury to persons in/or around the magnet, the force exerted by the magnetic field can cause metallic implants to move, thereby causing injury, current induced in an implant can cause local heating of tissue, magnetic fields and radiofrequency fields can interrupt the activity of electromagnetic implants, and radiofrequency power deposition may cause local heating of tissue. To protect against or treat these adverse effects, all subjects will be carefully screened for MRI contraindications using the standard MRI screening form, which includes questions about implants.These risks will be further minimized by strict adherence to standard MR operating procedures that require all ferromagnetic objects be kept from the magnet room and all subjects be screened for metallic objects and implants, and electromagnetic implants. Subjects will be required to remove metal objects from their person. Those with metallic or electromagnetic implants will be excluded from the study. The possibility of local tissue heating due to radiofrequency power deposition is mainly restricted to fast imaging protocols. The system automatically calculates and monitors power prior to and during the MR exam for each subject. Safety features of the software prevent starting an imaging protocol that exceeds FDA guidelines for power deposition. This safety feature is not subject to modification by researchers.The participant will be asked to wear earplugs or earphones while in the magnet.

Subjects may experience a feeling of being isolated or confined by the scanner (i.e., claustrophobia). We minimize these discomforts by explaining the procedure in great detail and by maintaining voice contact with the subject at all times. The most likely difficulty will be that a subject is unable to hold still for the MR scan. This would degrade the quality of the image obtained, but it would not be dangerous for the subject.

d) Adequacy of Protection against Risks

We will make every attempt to minimize all study-related risks. Women with reproductive potential must agree to the use of medically approved birth control, which includes condoms, oral contraceptives, diaphragms, and intrauterine device, during the study. Pregnant and nursing women will be excluded. We will test for pregnancy at each visit. We will carefully monitor for side effects and psychiatric symptoms at weekly visits throughout the study. In addition, complete blood count (CBC) is drawn at baseline, midpoint, and endpoint of study to monitor for any changes in blood count.

A partial HIPPA waiver will be obtained to permit the identification of potential participants through chart review. In addition, participants may be referred by their treatment team for consideration of study participation. No potential participant will be approached for recruitment without approval of a primary clinician, who will determine suitability of the person for the protocol. A chart review will be completed for all potential participants to reduce the likelihood that they will be found ineligible after participating in more extensive assessment. The study recruiter will verify with the primary clinician that a potential participant is sufficiently stabilized to consider participation and has capacity to provide consent. This is done prior to the study recruiter approaching a potential participant. The study recruiter will be introduced to the person and provide a brief overview of the project. Research staff members are trained to recognize symptoms of severe mental illness and cognitive impairment that could undermine an individual's ability to provide informed consent. Interested people will be provided study information and an informed consent form that contains all pertinent details of participation and includes the following: a brief explanation of the purpose of the research and a brief explanation of the requirements of the participant, including: a) willingness to be randomly assigned to either intervention, b) completing a series of interviews about one's symptoms, c) completing assessment tasks, and d) being available for follow-up assessments.

Participants will remain on adjunctive anticholinergics, antidepressants, mood stabilizers, and/or antianxiety agents. Participants on such medications are included to increase the generalizability (external validity) of study results and to relate the study more closely to treatment issues addressed in usual clinical practice. In order to decrease the likelihood that any observed change in outcomes during the study is related to adjunctive treatment, a participant must have been on the adjunctive medication(s) for at least two months and on his/her current dose(s) of adjunctive medication(s) for at least one month prior to study participation. The dose of adjunctive medication will remain fixed throughout the study, except for required side effect-related adjustments in anticholinergic medications. If at any time throughout the study a participant's condition worsens after permissible dose adjustments are made, he/she will be withdrawn.

All participants will be closely monitored and seen weekly during the study. Either the medically accountable physician or the PI may determine that the participant is experiencing clinical worsening, defined as: the participant is judged to be experiencing an exacerbation of his/her illness; an increase of 3 or more points from baseline on a BPRS positive symptom item or hostility; an increase of 2 or more points from baseline on the CGI global severity rating or 2 or more increase on CDS suicidal rating or new onset suicidal thoughts as judged by one of the 5 questions with an answer of "yes" on the C-SSRS. We will work with treatment teams at all sites and we will have a 24-hour telephone number with research staff available to take the call; participants can call at any time with their concerns, and they will be instructed to call when they are experiencing suicidal thoughts or symptom worsening, in depression, mania or psychotic symptoms. Research staff may direct them immediately to an emergency department. In addition, study withdrawal will occur in case of a side effect which is,clinically significant, possibly life threatening or may interfere with the study procedures . Participants may opt to

withdraw at any time during the study. Once a participant is planned for study withdrawal, all end-of-study measures will be performed, if possible, including blood collection and ratings, and we will refer the participant to other treatment. If serious suicidality is observed, the study suicide prevention plan will be implemented immediately and the participant will be withdrawn from study. The suicide prevention plan consists of immediate psychiatric evaluation by a psychiatrist, implementation of a suicide prevention contract, provision of 24-hour access to a physician, referral to emergency services as necessary, and continued evaluation using the CDS and C-SSRS.

Protection from research interview and data-gathering risks. In previous studies with this target population, we developed procedures for conducting interviews in a manner that is sensitive to the needs of the participants and the emotional nature of the interviews, while maintaining high scientific standards. Several of the strategies that we used successfully to minimize potential distress for participants include: informing participants before the interviews about the topics that will be covered; reminding participants that they may choose not to answer certain questions or to terminate the interview at any time; taking breaks during the interview when the participant feels anxious or distressed. Interviewers will be trained in the recognition of, and appropriate response to (including the involvement of other professionals), specific kinds of participant negative responses.

Confidentiality. Careful procedures will be used to protect the privacy of participants and the confidentiality of the data. Names will only appear on consent forms and on a master list that links them with study ID numbers (different from medical record numbers). This list will be stored in locked files in a separate location from the data. At the conclusion of the project, the list will be destroyed, unless continuation is planned and approved by the Institutional Review Board. All data (whether on forms or electronic data files) will be collected, analyzed, and reported according to the study ID number and will contain no names or other personal identifiers. Paper-based data will be stored in locked files. Electronic data files reside on desktop computers and are password protected. All videotapes that are created for fidelity and reliability purposes will be coded with an ID number and kept in a locked storage area. At the end of the research study, all videotapes will be destroyed. Because this study is conducted under an IND, there is a potential that identifiable data may be shared with the US FDA, grant management staff, University of Maryland IRB or quality assurance staff as part of its auditing process.

Protection from project intervention risks. A medically accountable physician is available to see all study participants. All clinical and research staff will be watchful regarding the participant's status, and any concerns are discussed with study PIs and will be reported to the participant's treatment team at the recruitment site. Research staff members have strict procedures for responding to suicidal or homicidal thoughts or behaviors, including ensuring immediate follow-up with the participant's treatment team or the study PIs. It is made clear to participants that they are free to terminate participation in the project at any time and return to usual clinical services without any penalty. If it is clear that involvement in any of the project's interventions is contributing to the worsening of a participant's status, the clinical staff will discuss the meaning and implications of this exacerbation with the participant and the research staff.

Steps taken to minimize the risks for MRI.
Prior to MR imaging subjects are interviewed twice to screen for contraindications. Those with metallic or electromagnetic implants will be excluded from the study. These risks will be further minimized by strict adherence to standard MR operating procedures that require all

ferromagnetic objects be kept from the magnet room. Subjects will be required to remove metal objects from their person.

The MR system automatically calculates and monitors power prior to and during the MR exam for each subject. Safety features of the software prevent starting an imaging protocol that exceeds FDA guidelines for power deposition. This safety feature is not subject to modification by researchers. Last, the participant will be asked to wear earplugs or earphones while in the magnet.

Careful planning and observation of the volunteer's response to the MR scanning session will allow research team members to ensure volunteers do not endure discomfort. The volunteer will be reminded during the MR scan that they can be seen and heard at all times and can discontinue participation at any time without consequence prior to or during scanning.

Consenting:
The consent form will include an explanation of the risks and benefits of participation; assurances of confidentiality; and an explanation that participation is entirely voluntary, the decision to participate will in no way influence or restrict services at participating sites, and the participant is free to withdraw at any time without negative consequences. Potential participants will be informed that the drug used in this study is not known to be effective for the indication under investigation. As some potential participants will have poor reading skills, the consent form will be read aloud to all participants in tandem with their own silent reading of the document. The individual securing consent will review any points about which the participant is unclear, and the participant will be invited to ask questions as needed. Our research staff is carefully trained in strategies for interacting with people with severe mental illness, including speaking slowly and clearly, stopping to summarize frequently, and providing time for questions. They are all supervised by senior staff members. All participants who express willingness to provide consent will be queried about the consent form in order to ensure that they have adequate understanding of the study requirements. This questioning is performed systematically, and research staff members document that this review has been completed. After reading the consent, and before obtaining a signature, a brief questionnaire is administered to verify that the participant is competent to provide consent and has demonstrated comprehension of the consent document. This questionnaire is attached to the informed consent form and is completed immediately after explaining the informed consent form and before obtaining the participant's signature on the form. If the participant does not understand the consent form, the recruiter will try to explain points of confusion, and administer the questionnaire again. Those failing to answer the questions adequately will not be recruited into the study. The recruiter will also make a clinical judgment and not recruit participants who appear unable to grasp key aspects of the procedure. This approach, which requires a proactive demonstration on the part of the participant that they understand what is being requested, has been used extensively by investigators at the MPRC. Included participants must also be judged competent to consent by the Evaluation to Sign Consent (ESC) questionnaire (DeRenzo et al 1998). Per University of Maryland School of Medicine IRB regulations, a copy of the signed consent form is given to the participant, a copy is placed in the person's medical record, and the original is kept by the PI.

Research assistants obtaining informed consent will be experienced clinicians. They will receive detailed and standardized training as to how to obtain informed consent from people with serious mental illnesses. They will be observed obtaining informed consent from a study participant by senior staff prior to being allowed to enroll participants on their own.

e) Potential Benefits of the Proposed Research to Participants and Others

By participating in this study and receiving a closely observed treatment trial with an investigational medication, the participant may have improvement in variety of symptom domains. In addition, we may improve understanding of the role of immune responses in schizophrenia treatment. Participants receive l-THP free of charge and have close observation during the study.

There are several other potential benefits from participating in the study. Participants receive standard elements of treatment, but with a heavier emphasis on continuity of clinical care and close clinical monitoring for signs of exacerbation than is common in community treatment. By virtue of their participation in numerous assessments and evaluations necessary in a combined clinical/research endeavor, they will receive more attention than is ordinarily given. Their progress will be frequently and closely monitored from a variety of clinically relevant standpoints. In addition, participants will gain specific information concerning their neurological status and observations of treatment response which might guide their future pharmacologic treatment. Moreover, participants and their families receive considerable education about the participant's illness. Such knowledge can provide significant benefits by increasing the capacity of the participant and his/her family to monitor and seek early clinical attention for future manifestations of illness.

f) Importance of the Knowledge to be Gained

The importance of the knowledge gained outweighs any risks that may occur in this study. L-THP has been used in China for 40 years and has a good safety record. This medication may provide an efficacious treatment for symptoms of schizophrenia and could provide benefits to millions of people with schizophrenia if found to be effective.

2. Inclusion of Women

The sample will consist of approximately 20 women. This one-third proportion of women is typical of previous research studies at MPRC.

3. Inclusion of Minorities

Racial data will be considered prior to ethnicity for the purpose of this study. Participants are permitted, however, to select more than one racial category. We expect about 32 (53% of total) participants to be African American, based on a 54% population of African Americans enrolled in previous clinical trials at the MPRC.

4. Inclusion of Children

Only those 18 and older will be included in this study because of limited safety data with l-THP in children and the different clinical nature of childhood schizophrenia.

5. Data and Safety Monitoring Plan

The Data Safety and Monitoring Board (DSMB) already established at the MPRC will be used for this study. The DSMB is comprised of three psychiatrists (2 involved in treatment and research, 1 who is a clinical practitioner), a pharmacist, and a statistician. The psychiatrists are experts in the clinical treatment of people with schizophrenia. The DSMB will be charged with

the following responsibilities:1)to establish a regular meeting schedule; 2) to review the protocol; 3) to review the consent form; 4) to monitor the occurrence of side effects/adverse events, and serious adverse events throughout the course of the study; and 5) to review with investigators, the study data management system; and 6) to establish stop rules for the study as a whole. The DSMB will review prior to study enrollment and every 6 months there after. In addition the DSMB will review the study once 20 participants have completed the study. The DSMB will receive bi-annual side effect/adverse event updates. All serious adverse events (SAEs) will be reported to the DSMB, PIs, and the University of Maryland School of Medicine IRB. The PIs will receive all SAE reports within 24 hours of their occurrence. If, as a result of data monitoring or interim analysis, the DSMB determines that the study poses an unreasonable or unnecessary risk to study participants, the DSMB and the PI will determine what protocol modifications are required to minimize the future occurrence of such events. Unexpected adverse events will be reported in accord with federal requirements. Non-serious and expected adverse events will be reported annually to the IRB. The PI is invited to the DSMB meetings and is asked to give a review of the past 6 months with a particular emphasis on safety, side effects and enrollment. The data remain blinded unless risks to participants justify unblinding as required by DSMB, FDA or IRB. To safeguard confidentiality, data are presented in aggregate or are identified only by an ID number.

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Appendix Drug List

DRUG
Alprenolol
Amitylpline
Atomoxetine
Captopril
Codeine
Dexflenfluramine
Duloxetine
Fenfluramine
Fluoxetine
Fluvoxatine
Hydrocodone
Metoprolol
Metoclopromide

Ondansetron
Oxycodone
Paroxetine
Propoxyphene
Quinidine
Ritonavir
Venlafaxine