Nitrous Oxide - A Novel Therapy for Treatment-Resistant Bipolar Depression

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ABSTRACT

Bipolar disorder (BPD) is a highly prevalent psychiatric disorder characterized by alternating periods of elevated (mania) and depressed mood. Despite the preponderance of treatment studies demonstrating efficacy in the manic phase of bipolar disorder, descriptive studies demonstrate that the depressed phase of BPD dominates the long term clinical pathology in this disorder. Further, studies also demonstrate that a significant subset (as high as 40%) of patients with BPD do not respond to standard antidepressant/mood stabilization treatments in the depressed phase.

Recently, ketamine, an NMDA-receptor antagonist has been shown to rapidly reverse the symptoms of MDD, both in unipolar and bipolar depression. Our group has demonstrated that nitrous oxide, another NMDA-receptor antagonist, is effective in treating unipolar treatment-resistant depression. Nitrous oxide may have considerable therapeutic advantages, including a cleaner side-effect profile (devoid of psychotomimetic symptoms), and easier delivery (no intravenous access needed). Therefore, using the successful approach applied in unipolar depression, we propose conducting a pilot, randomized, placebo controlled double-blind trial in which treatment-resistant depressed BPD patients will receive either nitrous oxide or placebo in addition to standard medical therapy.

SYNOPSIS

Study Title	**Nitrous Oxide - A Novel Therapy for Treatment-Resistant Bipolar Depression**
Objective	This clinical trial will determine if nitrous oxide (laughing gas) is an efficacious treatment for treatment-resistant bipolar depression.
Study Period	Planned study duration per patient: 6-8 weeks Planned study duration: 3 years
Number of Patients	64
Study Drug	Nitrous oxide (laughing gas). This is an FDA-approved general anesthetic and sedative agent used in hospitals and dentist offices. The use of nitrous oxide to treat bipolar depression is off-label and outside of the approved indication. Nitrous oxide will be administered under supervision of an attending-level anesthesiologist and according to standards set by the American Society of Anesthesiologists.
Intervention	Patients will receive a titrated mix of either 50% nitrous oxide/50% oxygen or "placebo" (50% nitrogen [inert]/50% oxygen) for 1 hour.
Study Design	Prospective, randomized, placebo-controlled trial using the Sequential Parallel Comparison Design. In the **first stage**, patients will receive 3 randomized 1-hour treatments of either nitrous oxide (25% patients) or placebo (nitrogen in air; 75% patients) every other day (e.g., M,W,F). In the **second stage**, participants assigned to placebo in Stage 1 will be randomized to three 1-hour treatments every other day (e.g., M,W,F) for one week, to either nitrous oxide (50% patients) or placebo (50% patients). Participants assigned to nitrous oxide in Stage 1 will remain on nitrous oxide in Stage 2. Assessments will be performed prior to each treatment series (baseline) and approximately 24 hours after inhalation sessions #1 and #2, and approximately 72 hours after inhalation session #3. One and two week assessments of continued efficacy will be made for exploratory evaluation, but not included in the primary efficacy analysis.
Inclusion and Exclusion Criteria	Inclusion Criteria a) Adults 18-75 years of age b) Treatment-resistant bipolar depressive disorder without psychosis and a MADRS baseline score of >20. All patients must have history of being on a serum-verified (at time of entry into study) mood stabilizer [lithium at 0.5-1.2mEq/L; or valproic acid, 50-125mcg/ml] or another mood stabilizer verified through medical records for 4 weeks prior to entry into the study and remain on this mood stabilizer during the course of the trial. Further, subjects will have failed two adequate dose/duration antidepressant courses in their lifetime, including one in the current depressive episode (verified by Antidepressant Treatment History Form). c) Good command of the English language Exclusion Criteria a) Schizophrenia b) Schizoaffective disorder c) Obsessive-compulsive disorder or panic disorder d) Active or recent substance abuse or dependence (in remission at least 1 year prior to the study; exception = nicotine use disorders) e) A diagnosis of personality disorder that may interfere with the patient's ability to improve on nitrous oxide as determined by study investigator f) Acute medical illness that may pose subject at risk during nitrous oxide administration (neurological disorders or medical disorders including dementia, stroke, encephalopathy, Parkinson's Disease, brain tumors, multiple sclerosis, seizure disorder, severe cardiac disease, any disease known to affect drug metabolism and excretion, i.e. renal or liver disease) per P.I. discretion g) Active suicidal intention (inability to contract for safety) h) Active psychotic symptoms i) Patients with significant pulmonary disease and/or requiring supplemental oxygen

	j) Contraindication against the use of nitrous oxide: pneumothorax; bowel obstruction; middle ear occlusion; elevated intracranial pressure; chronic cobalamin and/or folate deficiency treated with folic acid or vitamin B_{12}; pregnant patients; breastfeeding women; previous administration of NMDA-receptor antagonists (e.g., ketamine); current electro-convulsive therapy treatment
Primary Outcome	Change in depressive symptoms on MADRS scale between baseline and day 7 follow-up
Measurements	a) MADRS; b) Ham-D17; c) Young Mania Rating Scale (YMRS); d) Profile of Mood States Brief, 2nd edition (POMS 2); e) Quick Inventory of Depressive Symptoms-Self Report (QIDS –SR); f) Brief Psychiatric Rating Scale (BPRS); g) Scale for Suicidal Ideation (SSI); h) Clinician Administered Dissociative States Scale (CADSS)
Statistical Methodology	Comparison of scores on MADRS by repeated-measures mixed model

1. Specific Aims

1.1. To determine if nitrous oxide is an efficacious treatment for treatment-resistant bipolar depression.

1.2. To obtain data about the tolerability of nitrous oxide in this setting.

2. Background

Bipolar Disorder (BPD) is characterized by alternating periods of sustained elevation in mood (manic episodes) and decreased mood (depressive episodes) intermixed with euthymia. Historically, considerable research effort has been put towards treating the manic phase of BPD, while relatively little emphasis has been placed on the depressed phase of the illness (1, 2). While the emphasis has been on the manic phase of BPD, phenomenology studies of BPD demonstrate that the depressed phase predominates the overall time spent in mood dysregulation and is responsible for more psychosocial impairment (3, 4). Further, evidence demonstrates that a significant percentage (as high as 40%) of BPD patients suffering from the depressive phase are treatment resistant (5-7). In fact, meta-analyses suggest that in the depressed phase approximately 25% of BPD patients will respond to the addition of an adjunctive antidepressant (8).

Recently ketamine, an antagonist of the N-methyl-d-aspartate (NMDA) subtype of glutamate receptors, has been shown to produce a rapid antidepressant effect in patients with treatment-resistant unipolar depression after a single infusion of 0.5 mg/kg over 40 minutes (9-11). More recently, two studies have demonstrated similar antidepressant effects of ketamine in treatment resistant bipolar depression (12, 13).

Although these ketamine trials offer substantial promise in treating treatment-resistant bipolar depression, ketamine has several well-established side effects that may limit its clinical usefulness. These include dissociative symptoms, hallucinations, delusions, the possibility of precipitating mania in patients with BPD and significant sympathetic nervous system activation. In addition, administration of ketamine typically requires intravenous access and supervision by a physician experienced in sedation. These side effects render ketamine a less than ideal antidepressant, despite its favorable effects.

Preliminary Data

Nitrous oxide, commonly known as laughing gas, is the oldest and most commonly used anesthetic. It has a well-known safety profile and is widely and safely used as a sedative and analgesic by dentists. Similar to ketamine, nitrous oxide acts as an NMDA-receptor antagonist (14-16).

We hypothesized that nitrous oxide may have rapid antidepressant effects in patients with unipolar treatment-resistant major depression. In the first in-human, proof-of-principle, blinded, randomized placebo-controlled crossover trial (Nagele *et al*., Biological Psychiatry, 2015), 20 unipolar TRD patients received a 1-hour inhalation of 50% nitrous oxide or placebo. We found that depressive symptoms improved significantly after receiving nitrous oxide compared to placebo (mean HDRS-21

change at 2 hours: -4.8 points, 95% CI -1.8 to -7.8 points, p= 0.002; at 24 hours: -5.5 points, 95% CI -2.5 to -8.5 points, p<0.001; comparison between nitrous oxide and placebo: p<0.001). Four patients (20%) had treatment response and three patients (15%) a full remission after nitrous oxide compared to one patient (5%) and none after placebo (odds ratio [OR] for response 4.0, 95% CI 0.45 – 35.79; OR for remission 3.0, 95% CI 0.31 – 28.8, respectively). No serious adverse events occurred and all adverse events were brief and of mild to moderate severity. *These preliminary data strongly support our hypothesis that nitrous oxide will have antidepressant efficacy in patients with bipolar depression.*

Over the course of the past seven years, we have established the Washington University Treatment-Resistant Depression Clinic headed by Dr. Charles Conway. During this time, we have evaluated >125 patients with unipolar and bipolar treatment-resistant depression. Initial analyses of these data piqued our interest in the potential critical role of bipolarity in this population; we have observed that a large subset of treatment-refractory depression patients has significant family history of bipolarity (Conway et al., J Clin Psychiatry, 2015, in press), and of which a significant number may be responsive to nitrous oxide.

3. Drug Information

Nitrous oxide (laughing gas) is a colorless, odorless gas. Nitrous oxide is the oldest and most widely used FDA-approved anesthetic gas. The onset as well as offset of effect is within a few minutes. In this study we will use a mix of 50% nitrous oxide and 50% oxygen (nitrous) or 50% nitrogen/50% oxygen (placebo). This concentration (commonly used by dentists) avoids hypoxia and ideally achieves only mild to moderate sedation and is not sufficient to produce general anesthesia. Well-known side effects of nitrous oxide include: euphoria, sedation, nausea and vomiting, and inactivation of vitamin B_{12} (commensurate with the duration of exposure and concentration used). Exposure to 50% nitrous oxide for 1 hour is considered extremely safe and has been well-tolerated in our preliminary studies. The use of nitrous oxide to treat treatment-resistant bipolar depression is off-label.

4. Eligibility

4.1. *Inclusion Criteria*

a) Adults 18-75 years of age

b) Treatment-resistant bipolar depressive disorder without psychosis and a Montgomery-Asberg Depression Rating Scale (MADRS) baseline score of >20. All patients must have history of being on a serum-verified (at time of entry into study) mood stabilizer [lithium at 0.5-1.2mEq/L; or valproic acid, 50-125mcg/ml] or another mood stabilizer verified through medical records for 4 weeks prior to entry into the study and remain on this mood stabilizer during the course of the trial. Further, subjects will also have failed two adequate dose/duration antidepressant courses in their lifetime, including one in the current depressive episode (verified by Antidepressant Treatment History Form).

c) Good command of the English language

4.2. *Exclusion Criteria*

a) Schizophrenia

b) Schizoaffective disorder

c) Obsessive-compulsive disorder or panic disorder

d) Active or recent substance abuse or dependence (in remission at least 1 year prior to the study; exception = nicotine use disorders)

e) A diagnosis of personality disorder (confirmed by patient interview and medical chart review) that may interfere with the patient's ability to improve on nitrous oxide as determined by study investigator

f) Acute medical illness that may pose subject at risk during nitrous oxide administration (neurological disorders or medical disorders including dementia, stroke, encephalopathy Parkinson's Disease, brain tumors, multiple sclerosis, seizure disorder, severe cardiac disease,

any disease known to affect drug metabolism and excretion, i.e. renal or liver disease) per P.I. discretion

g) Active suicidal intention (inability to contract for safety)

h) Active psychotic symptoms

i) Patients with significant pulmonary disease and/or requiring supplemental oxygen

j) Contraindication against the use of nitrous oxide: pneumothorax; bowel obstruction; middle ear occlusion; elevated intracranial pressure; chronic cobalamin and/or folate deficiency treated with folic acid or vitamin B_{12}; pregnant patients; breastfeeding women; previous administration of NMDA-receptor antagonists (e.g., ketamine); current electroconvulsive therapy treatment

5. Enrollment

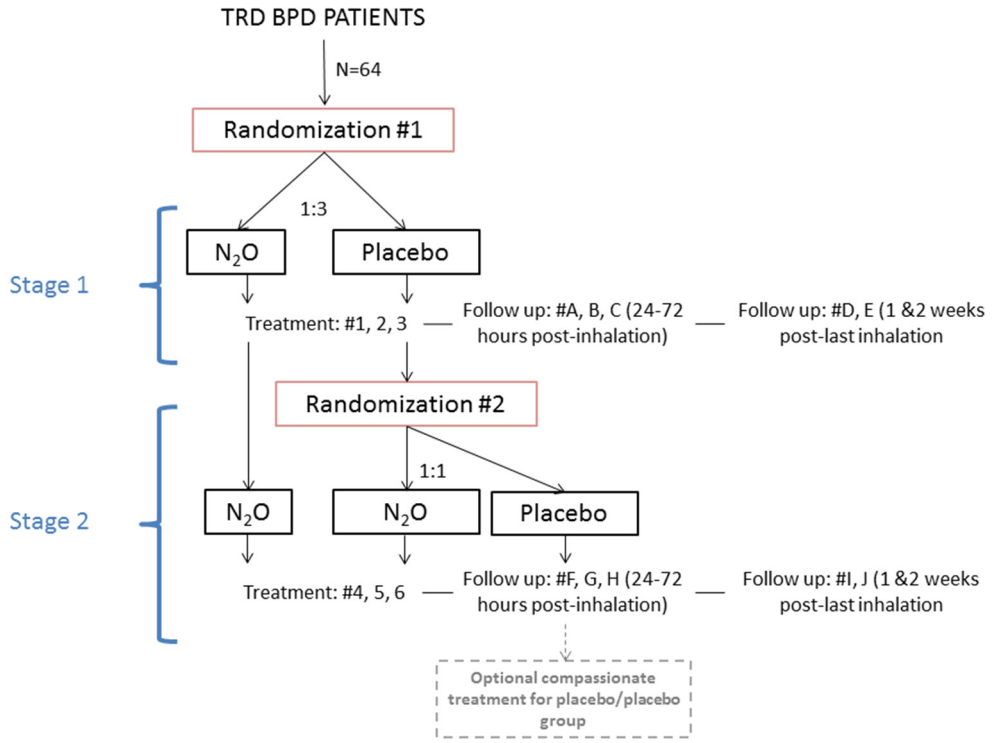
Patients with a diagnosis of bipolar depression and history of resistance to treatment (as defined above) will be recruited. Patients will be screened, consented for participation and enrolled in the study by study team personnel. We aim to recruit a total of 64 patients with the diagnosis of treatment-resistant bipolar depression, defined as failure to respond to two or more adequate dose-duration trials of antidepressant medications by history and chart verification.

6. Methods

6.1. Study Design (see summary figure below)

Prospective, randomized, placebo-controlled trial using the Sequential Parallel Comparison Design (SPCD) designed by PPD, a contract research organization. In the **first stage**, patients will be randomized (1:3 active [nitrous oxide] to placebo) to receive three 1-hour inhalation treatments every other day. Approximately 24-72 hours after the treatment session, patients will be assessed by the MADRS along with other scales as detailed below. Participants will also have follow-up visits/assessments performed approximately 1 and 2 weeks from the last inhalation session. The active treatment recipients from the **first stage** will receive a **second stage** of active treatment. All patients on placebo in the first stage ($n \approx 48$) will be re-randomized in the **second stage** (1:1 active to placebo) to three 1-hour treatments every other day, with the same follow-up visits/assessments as in Stage 1 (see study flow figure below). Throughout both stages group assignment will remain double-blinded to the participants and study raters conducting the assessments.

For ethical purposes, once the study blind has been broken those TRD subjects who were randomly assigned to receive successive placebo treatments will be offered an optional open label phase of active treatment (i.e., three 1-hour every other day inhalation treatments).



6.2. Randomization

The group assignment will be randomly assigned (1:3 active treatment to placebo) for each participant in Stage 1 and 1:1 (active: placebo) in Stage 2 for those previously assigned to placebo. Participants randomly assigned to active treatment in Stage 1 will remain on active treatment in Stage 2. PPD, LLC will be aiding in the randomization of participants. Study raters will remain blinded along with the participants to group assignment until the end of Stage 2. The team responsible for the administration of the nitrous oxide treatment and other study team members not conducting ratings will know the group assignment and can provide this information to participants or other team members if needed (see below for more information in 6.3).

6.3. Blinding

Patients will be blinded to the group assignment. Nitrous oxide is a colorless and odorless gas which makes it unlikely for patients to identify the group assignment. Likewise, the study setup will be identical for both sessions, which will make an inadvertent unblinding of the study unlikely. The team responsible for the administration of the nitrous oxide treatment will be aware of the treatment assignment. This team is completely separate from the team assessing the study outcomes, which will be blinded to the group assignment. After the completion of each stage, patients will be asked to assess if they received active treatment or placebo using a Blinding Questionnaire. Responses will be recorded with a 5 point scale asking participants to rate the extent to which they knew they were exposed to nitrous oxide; 1) strongly believe the treatment was nitrous oxide, 2) somewhat believe the treatment was nitrous oxide, 3) somewhat believe the treatment was placebo, 4) strongly believe the treatment is placebo, and, 5) don't know.

6.4. Setting

The study will be performed in ECT suite at Barnes-Jewish Hospital or The Clinical Research Unit (CRU) suite within Washington University. The procedure room is equipped with vital sign monitoring and resuscitation equipment and devices and oxygen wall outlets. As part of the standard setup, a mobile FDA-approved breathing circuit will be used for this study or a nitrous oxide tank will be attached to the anesthesia machine.

6.5. Administration of Study Treatment

Except for the choice of gas mix (nitrous oxide or nitrogen [inert] placebo; both mixed with 50% oxygen) treatment sessions will be identical. The gas mix will be administered via a standard anesthesia facemask through tubing connected to the anesthesia machine or via a facemask which is connected via hose to an FDA-approved Porter/Praxair MXR breathing circuit. Total gas flow will be 2-8 L/min and nitrous oxide concentration will be titrated over the first 10 minutes to a maximum of 50%. Patients will be monitored during and after the treatment according to American Society of Anesthesiologists standard which include continuous 3-lead ECG, pulse oximetry, non-invasive blood pressure and end tidal CO_2 under the supervision of an attending-level anesthesiologist. After the one-hour treatment session, patients will be monitored for approximately an hour. A study team physician will determine if the patient meets criteria for discharge before the patient will be cleared to leave the research suite.

7. Data Collection and Analysis

7.1. Follow-Up

Assessments will be performed before and after (approximately 1 and 24 hours) each treatment, with the exception of the 3rd inhalation treatments in each Stage which will be monitored at approximately 72 hours from last inhalation session. Additional follow-up visits will be scheduled approximately one and two weeks following completion of the final gas inhalation session in any study stage (to assess for lasting efficacy of nitrous effects). However, only the two 24 hour and one 72 hour measures will be used in the statistical analysis of efficacy. The one and two week assessments will be performed to obtain preliminary information regarding lasting efficacy.

7.2. Assessment of Treatment Efficacy

The effects of the treatment will be assessed using standard scales used for mood assessments in psychiatry, psychosis, and disassociation:

a) the MADRS (**Primary Outcome**) to assess change in depression;
b) the Hamilton Depression Rating Scale-17 item (Ham-D17), secondary measure of depression;
c) Young Mania Rating Scale (YMRS) to assess emergence of mania/hypomania (patients with YMRS scores > 12 will be removed from the trial and recommended for follow-up treatment per Psychiatry P.I., i.e., nitrous oxide treatments discontinued);
d) the Profile of Mood States, 2nd edition (POMS 2) to assess for rapid/instantaneous changes in mood/affect;
e) the Quick Inventory of Depressive Symptomatology- Self Report (QIDS –SR) patient-rated depression measure;
f) Clinician Administered Dissociative States Scale (CADSS) for emergence of disassociation;
g) the Brief Psychiatric Rating Scale (BPRS) to assess psychotic or hallucinogenic behavior; and
h) Scale for Suicidal Ideation (SSI) to assesses for emergence of suicidal thinking.

Patients will be assessed approximately 30 minutes before treatment, and approximately 1 hour and 24-72 hours after treatment by one of the expert psychiatry study team members who will be blinded to the study group assignment.

7.3. Assessment of Treatment Safety

Treatment safety will be assessed at several levels:

(1) Psychiatric AEs, such as psychotic symptoms or suicidal ideation, will be assessed via clinical interview as well as via standard scales. Emergence of psychosis will be identified by an increase in psychotic symptoms on the BPRS (for psychotic symptoms); suicidal ideation/intention will be assessed clinically and via the suicide question on the QIDS-SR (question #3) and the Scale for Suicidal Ideation (SSI) and recommended for additional follow-up treatment per Psychiatry P.I.

(2) Cardiovascular, respiratory and other AEs, will be identified clinically by continuous monitoring during and after the treatment. Follow-up recommendations will be given as needed, if necessary, by study personnel.

8. Data and Safety Monitoring

8.1. In general, the PIs have developed a specific set of Standard Operating Procedures (SOPs) for clinical research. All individuals working under the PI are required to read and be familiar and compliant with the SOPs. The PI's SOPs are in part developed from and are compliant with the Institutional guidelines, including those for a) Interactions with the Washington University Human Research Protection Office, b) Informed Consent Development and Implementation, c) Subject Recruitment and Screening, d) Subject Management While on Study, and e) Adverse Event Reporting.

8.2. The specific monitoring plan for this study is commensurate with the risks and the size and complexity of the investigations planned. The potential risks are attributable to the use of nitrous oxide and the underlying condition (bipolar depression). Based on these considerations the monitoring plan involves a **full DSMB** including an attending anesthesiologist knowledgeable in nitrous oxide pharmacology, an attending psychiatrist knowledgeable with an expertise in bipolar depression, and a biostatistician. All three members are not part of the study team. All reports of a Serious Adverse Event or an Unexpected Adverse Event will be investigated by the DSMB and reported to the IRB.

9. Data Analysis

The primary outcome variable is the change from baseline in MADRS scores during the first study week in each Stage of the SPCD study. Stage-wise test statistics representing the active vs. placebo response will be combined with equal weights for each Stage. The full analysis set is defined as all patients who were randomized and had at least one treatment dose as well as one post-baseline efficacy assessment in Stage 1. In Stage 2, the placebo non-responders who were re-randomized and had at least one treatment dose as well as one post-baseline efficacy assessment contribute to the full analysis set. A mixed model for repeated measurements (MMRM) with an unstructured variance-covariance matrix will be used for the primary efficacy analysis. This direct likelihood model is the missing data handling method recommended by the National Research Council of the National Academies of Science report on prevention and handling of missing data.

Secondary outcomes, expressed as change from baseline at 72 hours after the 3rd and final inhalation treatment will also be evaluated using the data from the full analysis set, consisting of all-comer participants from **Stage 1** and placebo non-responders in **Stage 2.** The secondary efficacy endpoints include changes in Ham-D17, Young Mania Rating Scale (YMRS), POMS, 2nd edition, and QIDS – SR. Intra- and inter-individual comparison of scores on depression scales will be done using a repeated-measures mixed effects model.

Based on our pilot study where we saw a significant reduction in depression scores among 20 unipolar depressed patients, we decided to enroll 64 patients in this trial which is similar (or larger) than previous studies of rapid antidepressants in this patient population.

10. Risk Assessment

10.1. Nitrous oxide

The side effects of nitrous oxide are well known to anesthesiologists. The dose (50%) and duration (1 hour) used in this protocol are considered very safe and low risk. Similar doses and durations are used in everyday dental practice without vital sign monitoring or supervision by a resuscitation-trained physician. With this dose of nitrous oxide, sedation may occur but not general anesthesia or respiratory depression. Minor side effects after nitrous oxide exposure such as nausea and vomiting may occur (and were observed in our preliminary study in unipolar depression), which are typically

self-limited and short. If a patient develops moderate to severe nausea and vomiting, we may administer 4mg of ondansetron. Other risks include euphoria, anxiety and increased heart rate.

10.2. Major depression and suicide risk
Because this is a first in-human study of nitrous oxide in the treatment of bipolar depression, a worsening of depression symptoms and an increased risk for suicide cannot a priori be excluded. After each treatment, an expert research team member and, if needed, psychiatrist will determine the suicide risk and if indicated, take the necessary precautions to mitigate this risk including recommendation of treatment, hospitalization and withdrawal of subject from the study to preserve safety.

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