Study Protocol

Inhaled Nitrous Oxide for Treatment-Resistant Depression: Optimizing Dosing Strategies

Protocol Version:
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5/24/2019

Principal Investigator:

Ben Palanca, MD PhD
Assistant Professor of Anesthesiology
Department of Anesthesiology, Campus Box 8054
Phone: 314-362-7823
Email: palancab@wustl.edu

Co-Investigator:

Peter Nagele, MD, MSc
Professor of Anesthesiology
Department of Anesthesiology & Critical Care,
University of Chicago
Email: nagelep@uchicago.edu

Charles Conway, MD
Professor of Psychiatry
Department of Psychiatry, Campus Box 8134
Phone: 314-362-2463
Email: conwayc@psychiatry.wustl.edu

ABSTRACT

Most clinical major depression responds to standard treatments (medication and psychotherapy); however, a significant subset of depressed patients (15-20%) do not respond to these treatments and are referred to as treatment-resistant major depression (TRMD). New treatments for TRMD are needed, and one promising line of research are drugs known as N-methyl-D-aspartate (NMDA) glutamate receptor antagonists. In a recent pilot study, our group demonstrated that the NMDA antagonist nitrous oxide (N_2O) is effective in TRMD, however, the dose concentration of nitrous oxide to have antidepressant effects is unknown. Therefore, we propose conducting a randomized, placebo controlled double-blind crossover (x2) study in which treatment-resistant depression patients will all receive 3 randomized, one hour nitrous oxide inhalation sessions of placebo (0% N_2O), 25% N_2O (low dose), and 50% N_2O (high dose). We aim to compare nitrous oxide's relative antidepressant efficacy of two doses of N_2O by demonstrating that inhaled nitrous oxide, but not inhaled placebo, will significantly show rapid and sustained antidepressant effects.

SYNOPSIS

Study Title	Inhaled Nitrous Oxide for Treatment-Resistant Depression: Optimizing Dosing Strategies
Objective	This study aims to determine whether different concentrations of nitrous oxide (N_2O) have different antidepressant effects for adults with treatment-resistant major depression.
Study Period	Planned enrollment duration: Approximately 2 years Planned study duration: Approximately 18 weeks
Number of Patients	40
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 in this protocol is off-label and outside of the approved indication. Nitrous oxide will be administered under supervision of an attending-level anesthesiologist and using standard anesthesia equipment (mobile anesthesia machine/FDA-approved nitrous oxide delivery system, standard monitoring, oxygen, rescue equipment).
Study Design	Prospective, randomized, placebo-controlled, cross-over (x2) trial. All patients will receive 3 randomized, one hour N_2O inhalations to placebo (0% N_2O), low dose (25% N_2O), and high dose (50% N_2O). Inhalation sessions will be at least 4 weeks apart. Mood will be assessed at baseline, 24 hours, and 1, 2, and 4 weeks post-inhalation for each dose.
Inclusion and Exclusion Criteria	<u>Inclusion Criteria</u> a) Adults 18-75 years of age; b) Current diagnosis of unipolar major depressive disorder (MDD) without psychosis as confirmed by structured clinical interview for DSM-IV disorders; c) A score of ≥ 9 on the Montgomery-Åsberg Depression Rating Scale (MADRS); d) Documented (i.e., chart review) lifetime failure to respond to ≥3 adequate dose/duration antidepressant treatment trials, ≥1 medication failure in the current depressive episode; e) Good command of the English language. <u>Exclusion Criteria</u> a) Meets criteria for any DSM-IV diagnosis for schizophrenia, bipolar, schizoaffective, obsessive-compulsive, personality, or panic disorders; b) Any recent (within past 12 months) history of substance dependence or abuse (except tobacco), determined by reported history and urine drug screen; c) Ability to become pregnant and not using effective contraception; d) Contraindication against the use of nitrous oxide: 1) Pneumothorax 2) Bowel obstruction 3) Middle ear occlusion 4) Elevated intracranial pressure 5) Chronic cobalamin and/or folate deficiency treated with folic acid or vitamin B12 6) Pregnant patients 7) Breastfeeding women e) Inability to provide informed consent; f) Any other factor that in the investigators' judgment may affect patient safety or compliance
Primary Outcome	Comparison of change in Hamilton Depression Rating Scale score at immediate (2 hrs) and sustained (24hrs) post nitrous oxide inhalation. Secondary depression measures will be assessed at the later time points for duration of sustained antidepressant effects.
Measurements	Hamilton Depression Rating Scale, HDRS-21 scale; Montgomery-Åsberg Depression Rating Scale, MADRS; Quick Inventory of Depressive Symptomology-Self Report, QIDS-SR; Clinician Administered Dissociative States Scale, CADSS-28 item; Brief Psychiatric Rating Scale, BPRS; Profile of Mood States, POMS-2, MINI International Neuropsychiatric Interview. Electroencephalography (EEG) during eyes open and eyes closed periods.
Statistical Methodology	Outcomes will be analyzed with a repeated measures mixed effects linear model using restricted maximum likelihood estimation. To adjust for the observed carryover effect, the model will include a randomization group term and a three-way interaction. Response and remission rates will be compared with an exact binomial test (and corresponding odds ratios calculated).

1. Specific Aims

1.1. To demonstrate that both low and high concentrations of nitrous oxide will show equivalent and superior antidepressant effects relative to placebo at immediate (2 hours) and sustained (24 hours) time points.

1.2. To compare the sustained antidepressant effect of low and high concentrations of inhaled nitrous oxide at 1, 2, and 4 weeks post-inhalation. We hypothesize that for both low and high doses, antidepressant effects will be attenuated at 1 and 2 weeks, and will dissipate by one month.

2. Background

It is estimated that as many as 16 million Americans suffer from major depressive disorder (MDD). Fifty percent fail an initial pharmacotherapy trial and ~30% fail a series of treatment trials [1], and are referred to as "Treatment-Resistant Major Depression" (TRMD). TRMD exacts a high toll on treatment resources and studies suggest that patients with TRMD place an unusually heavy burden on total MDD costs (suicide, personal financial costs, loss of work, medical co-morbidity [2, 3]). Unfortunately, knowledge regarding the best treatments for TRMD remains incomplete.

Studies demonstrate that there are limited effective antidepressant treatments for TRMD. Evidence shows that only four treatment approaches are effective in MDD after two sequential ineffective antidepressant trials: lithium augmentation, monoamine oxidase inhibitors, electroconvulsive therapy, and atypical antipsychotics (e.g. aripiprazole, quetiapine; [3]). To address this problem, the NIMH sponsored the largest sequential antidepressant trial (STAR*D). Following four levels of adequate dose-duration medications, ~36% of patients failed to remit [1]. *The STAR*D trial concluded that a significant proportion of MDD patients do NOT achieve remission and constitute TRMD.* With few exceptions, the majority of existing antidepressant treatments act via mechanisms discovered over three decades ago. ***Hence, antidepressant treatments with alternate mechanisms of action are needed.***

Over the past 15 years, multiple studies have indicated that the NMDA antagonist ketamine is an effective, rapid onset antidepressant in TRMD [4]. Most noteworthy, these studies suggest that ketamine acts immediately, producing an antidepressant response in less than 24 hours- a distinct advantage to existing antidepressant medications, which typically take 4-6 weeks to effectively decrease depressive symptoms. Despite the enormous potential benefits of ketamine in TRMD, this compound carries significant limitations: it is associated with psychosis, high addiction potential, and potential cognitive impairment [5-8].

Nitrous oxide (N_2O), an odorless, colorless gas typically used in anesthesia and dentistry, is an NMDA receptor antagonist [9]. Our group recently completed the first study of N_2O in TRMD patients [10]. Using a double-

blind, crossover design, we found that TRMD patients receiving a one hour session of inhaled N_2O (50% N_2O in 50% oxygen) versus placebo (50% nitrogen in 50% oxygen) experienced a significant decrease in MDD symptoms at 2 and 24 hours after inhalation, using the Hamilton Rating Scale for Depression-21 item (HDRS-21). Mean HDRS score differences at 2 hours: -4.8 points, p=0.002; and 24 hours: -5.5 points, p<0.001). Four patients (20%) had treatment response (HDRS reduction ≥50%) and three patients (15%) a full remission (HDRS ≤7 points) after N_2O, compared to one patient (5%) and none after placebo (odds ratio [OR] for response 4.0, 95% CI 0.45–35.8; OR for remission 3.0, 95% CI 0.31– 28.8). No serious adverse events occurred; however, *two patients experienced panic attacks/anxiety onset, suggesting that the inspiratory N_2O concentration may have been too high*. Importantly, the N_2O dose in our pilot study (50% N_2O in 50% oxygen) was based on its use in dentistry because no prior information about N_2O in TRMD existed.

Preclinical studies suggest that different doses of ketamine are associated with variable NMDA receptor and antidepressant activity. In single neurons, 50% N_2O blocks >50% of NMDA receptors, whereas ketamine concentrations achieved in depression studies likely block ~25-35% of NMDA responses [11]. Thus, it is possible that a lower inspiratory concentration of N_2O (25%) will have substantial antidepressant effect while also reducing the risk of paradoxical anxiety.

We propose a pilot study using a double-blind, crossover (x 2), placebo-controlled design to determine whether different concentrations of N_2O have different antidepressant effects. We hypothesize that 25% and 50% N_2O, as compared to placebo, will have equivalent antidepressant effects. Further, we predict that these effects will occur rapidly (2 hours) and be sustained (24 hours, one and two weeks), but will attenuate by one month.

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. It is commonly used as a component of general anesthesia and as a sedative/analgesic agent used in hospitals and dentist offices. The onset as well as offset of effect is within a few minutes. Nitrous oxide is the least potent of inhalational anesthetics. Concentrations above 1 atm are theoretically needed to produce complete general anesthesia when nitrous oxide is used as a sole agent. Therefore, concentrations typically used (50%) in dentistry and pediatrics achieve only mild to moderate sedation (but potent analgesia) and are not sufficient to produce general anesthesia. Well-known side effects include euphoria, sedation, nausea and vomiting, inactivation of vitamin B_{12} (commensurate with the duration of exposure and concentration used). In general, exposure to 25-50% nitrous oxide for 1 hour is considered extremely safe. The use of nitrous oxide in this protocol is off-label and outside of the approved indication.

4. Eligibility

4.1. Inclusion Criteria

a) Adults 18-75 years of age;

b) Current diagnosis of unipolar major depressive disorder (MDD) without psychosis as confirmed by structured clinical interview for DSM-IV disorders;

c) A score of ≥ 9 on the Montgomery-Åsberg Depression Rating Scale (MADRS);

d) Documented (i.e., chart review) lifetime failure to respond to ≥3 adequate dose/duration antidepressant treatment trials, ≥1 medication failure in the current depressive episode;

e) Good command of the English language.

Exclusion Criteria

a) Meets criteria for any DSM-IV diagnosis for schizophrenia, bipolar, schizoaffective, obsessive-compulsive, personality, or panic disorders;

b) Any recent (within past 12 months) history of substance dependence or abuse (except tobacco), determined by reported history or urine drug screen;

c) Ability to become pregnant and not using effective contraception;

d) Contraindication against the use of nitrous oxide:

1) Pneumothorax
2) Bowel obstruction
3) Middle ear occlusion
4) Elevated intracranial pressure
5) Chronic cobalamin and/or folate deficiency treated with folic acid or vitamin B12
6) Pregnant patients
7) Breastfeeding women

e) Inability to provide informed consent;

f) Any other factor that in the investigators' judgment may affect patient safety or compliance.

5. Enrollment

Participants will be recruited in part through the Volunteer for Health registry at Washington University School of Medicine and the Washington University TRMD Database (120+ individuals with TRMD carefully screened over the past 8 years, maintained by Dr. Conway; IRB# 201102247). Eligible and interested participants will be consented for participation and enrolled in the study by study personnel. We will recruit a total of 40 participants currently in an episode of major depressive disorder, medication-free or on psychotropic medications. To complete the study, we will enroll up to 40 participants to achieve adequate sample size.

6. Methods

6.1. Study Design

This is a double-blind, randomized placebo-controlled crossover trial in which all subjects will undergo three randomized, one hour N_2O inhalation sessions each separated by at least 4 weeks. Inhalations will include exposure to placebo (0% N_2O), 25% N_2O (low dose), and 50% N_2O (high dose). Mood will be assessed at baseline (immediate pre-treatment), immediate (1 hour post-inhalation), sustained (24 hours) and at one and two weeks, and one month. We will also assess for potential psychiatric side effects including psychosis and disassociation.

There will be up to 14 study visits involved with participation. The screening visit (Visit 1) will be used to verify eligibility and collect background information.

Specifically, we will collect patient demographic information, medical history, vital signs and a research team member will perform a physical exam. A urine sample will be collected for drug, and an optional blood draw may be collected if the study physician requests tests to confirm participants' safety to participate. Psychiatric assessments (completed by the participant and administered by the research team) will be performed to assess for major depression, other mental health diagnoses including significant anxiety disorder, post-traumatic stress disorder, and psychosis or dissociation.

Visit 2 may directly follow Visit 1 on the same day, if needed. Visit 2 (Baseline visit) will ask participants to fill out several mood-based questionnaires, pass a urine pregnancy screen (Baseline visit only), receive a total of 60 minutes gas inhalation, and after the observation period, will be asked several mood-based questionnaires again. Visit 3 is scheduled approximately 22-28 hours after completion of the inhalation session, and participants will have vital signs taken and mood questionnaires asked. Visits 4 and 5 will occur 1 and 2 week(s), respectively, after inhalation session one, and involve asking participants about their mood and mental state. Visit 6 will occur 4 weeks after inhalation session one, and will include questionnaires to account for a 4 week follow up and also a baseline visit for the next gas concentration. The procedures for Visit 6 will be identical to Visit 2 (besides urine pregnancy screen) with gas inhalation and mood-based questionnaires before and 1 hour after inhalation. The remaining follow up visits will follow the same outline until all 3 gas concentrations have been given to each participant and follow up visits 24 hours, and 1, 2, and 4 weeks after each inhalation session have been completed. If necessary, the 1, 2, and 4 week post inhalation session visits may be conducted over the phone with Principal Investigator approval.

Participants will be monitored after each inhalation session before being allowed to leave. Optional labs may be ordered by the screening physician to confirm the safety of a patient. These screening labs might include but are not limited to a comprehensive blood panel and a complete blood count.
Patients will be reimbursed with $5 + parking for each visit completed after the Screening Visit for their expenses and time commitment. If a participant completes all visits they will receive $65. Otherwise if the participant ends participation before the study visits are complete, the participant will be compensated $5 for each visit they did complete.

6.2. Randomization

The order of the 3 inhalation sessions will be randomly assigned for each patient by a random number generator.

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. For the team responsible for the administration of the nitrous oxide treatment, the group assignment will be known. This team is completely separate from the team assessing the study outcomes which will be blinded to the group assignment. Treatment administration and outcomes assessment may also be physically separated in different rooms within the study facility.

In addition, after each inhalation session, subjects will be asked whether they thought they received the active nitrous oxide gas or the placebo gas. Responses will be recorded with an assessment (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 inhalation sessions will be performed in Barnes-Jewish-Hospital, or the Clinical Research Unit (CRU) suite within Washington University. The procedure room used is equipped with vital sign monitoring, resuscitation equipment and devices, and has oxygen wall outlets. As part of the standard setup, a mobile FDA-approved nitrous oxide delivery system will be used or an anesthesia machine.

6.5. Study Procedures

Except for the choice of gas (either nitrous oxide concentration or nitrogen [inert]; all 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. A small sample connector line will be inserted into the facemask which allows the measurement of inhaled and exhaled gas concentrations. Inhaled and exhaled gas concentrations of nitrous oxide, oxygen and nitrogen will be monitored and adjusted according to protocol. Total gas flow will be 2-8 L/min.

Patients will be monitored during and after the treatment according to American Society of Anesthesiologists standard which include continuous 3-lead ECG, pulsoximetry, non-invasive blood pressure and endtidal CO_2 under the supervision of an attending-level anesthesiologist. After the one hour treatment session, patients may be transferred if needed, and monitored in a recovery room for approximately 2 hours. A study team physician will determine if the patient meets criteria for discharge before the patient will be allowed to leave the suite.

7. Data Collection

7.1. Follow-Up

Data will be examined from baseline (prior to each inhalation session) to immediate (1 hours post-inhalation) and sustained (24 hours post-inhalation) effects versus compared to each other and the placebo gas. Additional follow-up visits will be scheduled approximately 1, 2, and 4 weeks following completion of each gas inhalation session (to assess for treatment efficacy and also duration of efficacy of nitrous oxide effects).

7.2. Assessment of Treatment Efficacy

The effects of the inhalation gases on behavioral and mood state will be assessed using standard scales used in clinical psychiatry:

- Hamilton Depression Rating Scale 21 items (HDRS-21) scale (**Primary Outcome**)
- Montgomery-Åsberg Depression Rating Scale (MADRS)
- Quick Inventory of Depressive Symptomology-Self Report (QIDS-SR)
- Clinical Administered Dissociative States Scale 28 item (CADSS-28)
- Brief Psychiatric Rating Scale (BPRS)
- Profile of Mood States 2nd Edition (POMS-2)

Patients will be assessed at all time points by one of the psychiatry study team members who will be blinded to the study group allocation.

Additionally, adequacy of the blind will be assessed by a Blinding Questionnaire given after completion of each inhalation session (see Section 6.3).

7.3. Assessment of Treatment Safety

Treatment safety will be monitored at several levels: adverse events (AE) related to (i) cardiovascular status; (ii) respiratory function; (iii) central nervous system; and (iv) psychiatric symptoms, particularly the presence of psychotic symptoms.

(i) 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.

(ii) Psychiatric AEs, such as psychotic and dissociative symptoms, will be assessed via clinical interview as well as via standard scales before and after inhalation sessions. 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 questions on the QIDS-SR (question #3) and the HDRS-21 and recommended for additional follow-up treatment per Psychiatry P.I.

7.4. Sources of Research Material

Data may be obtained from medical records and hospital charts, as well as from data collected at each study visit from the research assessments administered either by study personnel interview or by participant self-report.

8. Data and Safety Monitoring

8.1. In general, the PI has 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 Subjects Review Committee, b) Informed Consent Development and Implementation, c) Subject Recruitment and Screening, d) Subject Management While on Study, 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 PI will review all study data on an ongoing basis. The potential risks are attributable to the use of the gas mixtures or emotional discomfort while completing the assessments/questionnaires.

9. Data Analysis

Major depressive disorder symptoms will be evaluated at baseline (pre-inhalation), at 1 and 24 hours, at one and two weeks, and one month post-inhalation. The primary MDD measure is the change in HDRS-21 scale at 2 and 24 hours post-inhalation. Later time points will assess for duration of sustained antidepressant effects (Aim 2).

Secondary MDD measures taken at the same time points, will include the MADRS, the QIDS-SR, and the POMS 2 (immediate subjective mood change). At the same time points, euphoria, psychosis (BPRS), and disassociation (CADSS) will also be measured. Outcomes will be analyzed with a repeated measures mixed effects linear model using restricted maximum likelihood estimation. To adjust for the observed carryover effect, the model will include a randomization group term and a three-way interaction (treatment x time x randomization group). To compare response and remission rates (≥50% decrease, ≤7 on HDRS-21, respectively), an exact binomial test will be used (and corresponding odds ratios [ORs] calculated). Symmetry and spectral power will be assessed from EEG acquired during periods of eye closure or opening.

Power Analysis

Our original pilot study in TRMD patients found statistical significance with 20 subjects. Based on the assumption that the low N2O concentration (25%) is non-inferior to the high N_2O concentration, 24 patients will be required with a power of 90% to show that the lower limit of a one-sided 95% confidence interval (or equivalently a 90% two-sided confidence interval) will be above the non-inferiority limit of -3 points on the HDRS with an expected SD of 2.5.

10. Risk Assessment

10.1. Nitrous Oxide

The side effects of nitrous oxide are well known to all anesthesiologists. The dose (25% or 50%) and duration (1 hour) used in this protocol are considered extremely safe and of low risk. Similar doses and durations are used in everyday dental practice without vital sign monitoring or supervision by a resuscitation-trained physician. The gas inhalation will be administered under supervision of an attending-level anesthesiologist and according to standards set by the American Society of Anesthesiologists, which includes monitoring via: 3-lead ECG, pulsoximetry, non-invasive blood pressure, and end-tidal CO_2. With this dose of nitrous oxide, sedation may occur but not general anesthesia or respiratory depression. Previous evidence shows that the inactivating effect of nitrous oxide on vitamin B_{12} is dose and duration-dependent and are minor when used as in this protocol. An increase in plasma homocysteine by +25% can be expected which is expected to revert to baseline within the next 24 hours. Minor side effects after nitrous oxide exposure such as nausea and vomiting may occur which are typically self-limited and short. If a patient develops moderate to severe nausea and vomiting, we may administer 4mg of ondansetron. After the inhalation treatment sessions, clinical and nursing staff will

continue to be present to monitor patients' symptoms and state before discharging the patient. Blood pressure and heart rate are also checked at the 22-28 hour after inhalation session follow-up visit.

10.2. Major depression and suicide risk

Because this is a novel study of nitrous oxide in the treatment of treatment-resistant 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.

10.3. Clinical Interview and Assessment

All clinical assessors have extensive experience in clinical psychiatric assessment and will make every effort to implement protocol procedures in a sensitive and supportive manner. Subjects will be given these assessments in a quiet, private setting with breaks given as needed. Other measures to minimize risks include the careful assessment of each subject before the study, and close clinical scrutiny during all aspects of the study.

11. References

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