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Nitrous Oxide for Treatment-Resistant Major Depression: A Proof-of-Concept Trial

Peter Nagele • Andreas Duma • Michael Kopec • J. Philip Miller • Charles F. Zorumski • Charles R. Conway • Show all authors

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Abstract

Background

N-methyl-D-aspartate receptor antagonists, such as ketamine, have rapid antidepressant effects in patients with treatment-resistant depression (TRD). We hypothesized that nitrous oxide, an inhalational general anesthetic and *N*-methyl-D-aspartate

receptor antagonist, may also be a rapidly acting treatment for TRD.

Methods

In this blinded, placebo-controlled crossover trial, 20 patients with TRD were randomly assigned to 1-hour inhalation of 50% nitrous oxide/50% oxygen or 50% nitrogen/50% oxygen (placebo control). The primary endpoint was the change on the 21-item Hamilton Depression Rating Scale (HDRS-21) 24 hours after treatment.

Results

Mean duration of nitrous oxide treatment was 55.6 ± 2.5 (SD) min at a median inspiratory concentration of 44% (interquartile range, 37%–45%). In two patients, nitrous oxide treatment was briefly interrupted, and the treatment was discontinued in three patients. Depressive symptoms improved significantly at 2 hours and 24 hours after receiving nitrous oxide compared with placebo (mean HDRS-21 difference at 2 hours, –4.8 points, 95% confidence interval [CI], –1.8 to –7.8 points, $p = .002$; at 24 hours, –5.5 points, 95% CI, –2.5 to –8.5 points, $p < .001$; comparison between nitrous oxide and placebo, $p < .001$). Four patients (20%) had treatment response (reduction ≥50% on HDRS-21) and three patients (15%) had a full remission (HDRS-21 ≤ 7 points) after nitrous oxide compared with one patient (5%) and none after placebo (odds ratio for response, 4.0, 95% CI, .45–35.79; OR for remission, 3.0, 95% CI,

.31–28.8). No serious adverse events occurred; all adverse events were brief and of mild to moderate severity.

Conclusions

This proof-of-concept trial demonstrated that nitrous oxide has rapid and marked antidepressant effects in patients with TRD.

Keywords

Major depression • Nitrous oxide • Treatment-resistant depression

depression (estimated prevalence in the United States is 10 million adults) is affected (2). Therapeutic options for TRD are very limited.

There is a strong biological rationale supporting the potential therapeutic use of nitrous oxide in TRD. Although nitrous oxide is known to modulate several central nervous system targets (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14), like ketamine, the primary target of nitrous oxide appears to be the *N*-methyl-D-

aspartate (NMDA) receptor, where nitrous oxide acts as a noncompetitive inhibitor (15, 16, 17). NMDA receptor signaling has been implicated in the neurobiology of depression and is a key component of central nervous system information processing (18, 19, 20). Consistent with the relevance of NMDA receptor signaling in the pathophysiology of major depression, NMDA receptor antagonists, such as ketamine (a general, dissociative anesthetic), have been shown to provide rapid and sustained antidepressant effects at subanesthetic doses in TRD (21, 22, 23, 24, 25, 26, 27). Given the similar mechanisms of action, we hypothesized that nitrous oxide may also have rapid antidepressant effects in TRD. This proof-of-concept trial assessed the immediate (2 hours) and sustained (24 hours) antidepressant effects of nitrous oxide in a population of well-characterized patients with TRD.

Methods AND MATERIALS

Study Design and Oversight

This study was designed as a randomized, placebo-controlled crossover pilot clinical trial testing the antidepressant effects of nitrous oxide in 20 patients with TRD. In this study, patients had two treatment sessions that were 1 week apart (nitrous oxide or placebo). The sequential order of the sessions was

assigned by a random number generator. Other than the gas mixture administered, the sessions were indistinguishable in setting, setup, and monitoring.

We undertook several measures to ensure treatment blinding. First, we completely separated personnel and location of the team providing nitrous oxide treatment from the team performing psychiatric evaluations. The two locations were physically separated from each other, and no team member was allowed to enter the other space while a study patient was present. Second, records for the nitrous oxide and placebo treatment administration were kept separate from the psychiatric assessment case report forms until completion of the study. Third, all equipment used to provide treatments was identical between nitrous oxide and placebo sessions. Lastly, patients were blinded as to the nature of the inhaled gas at each inhalation session; all patients were informed that they would receive either nitrous oxide or an air mixture with a high nitrogen component (placebo).

A data and safety monitoring board monitored the trial. The study was approved by the Washington University in St. Louis Institutional Review Board, and all patients provided written, informed consent. The trial was registered at clinicaltrials.gov (NCT02139540).

Patients

Patients were recruited from an existing database of

patients with TRD administered by the Washington University Department of Psychiatry and from the "Volunteers for Health" patient pool (individuals with various medical or psychiatric conditions who volunteer to participate in clinical research) within Washington University School of Medicine. Inclusion criteria were 1) age 18–65 years; 2) meeting DSM-IV-TR criteria for major depressive disorder without psychosis, as determined using a structured clinical interview [Mini International Neuropsychiatric Interview (28)]; 3) a pretreatment score >18 on the 21-item Hamilton Depression Rating Scale (HDRS-21); and 4) meeting criteria for TRD, defined as having had at least two adequate dose-duration, antidepressant medication failures in the current depressive episode and a lifetime failure of at least three antidepressant medication trials. Exclusion criteria were 1) a history of bipolar disorder, schizophrenia, schizoaffective disorder, obsessive-compulsive disorder, panic disorder, or documented Axis II diagnoses; 2) active or recent substance abuse or dependence ("recent" defined as within the past 12 months; exception was made for nicotine use disorder); 3) the presence of acute medical illness that could interfere with study participation, including, but not limited to, significant pulmonary disease; 4) active suicidal intention; 5) active psychosis; 6) previous administration of NMDA receptor antagonists (e.g., ketamine); 7) ongoing treatment with electroconvulsive therapy; 8) pregnancy or breastfeeding in female patients; and

9) contraindications against the use of nitrous oxide (e.g., pneumothorax, middle ear occlusion, elevated intracranial pressure, chronic cobalamin or folate deficiency treated with folic acid or vitamin B_{12}). Patients were instructed to continue their current standard of care treatment for major depression and were required to maintain a stable medication or psychotherapy regimen without changes for 4 weeks before initiation of the study and to continue on the same dosage throughout the study.

Treatment

Patients received either an admixture of up to a maximum of 50% nitrous oxide and 50% oxygen ("active treatment") or 50% nitrogen/50% oxygen ("placebo") for 1 hour. The inspiratory nitrous oxide concentration was titrated during the first 10 min until 50% was achieved. The 50% nitrous oxide concentration was selected in this pilot trial based on clinical experience for sedation in dentistry and obstetric analgesia, where 50% nitrous oxide has been used for decades with an excellent safety and effectiveness record. We decided to maintain an equal oxygen concentration (50%) in the placebo treatment to limit the variability between treatment and placebo. The gas mix was administered via a standard anesthesia facemask through tubing connected to an anesthesia machine. A small sample connector line was inserted into the facemask allowing the measurement of inhaled and exhaled gas concentrations. Total gas flow was 4–8

L/min. Patients were monitored during and after the treatment according to the American Society of Anesthesiologists standard, which includes continuous three-lead electrocardiogram, pulse oximetry, noninvasive blood pressure, and end-tidal carbon dioxide under the supervision of an attending-level anesthesiologist. After the 1-hour treatment session, patients were transferred to a recovery area and monitored for 2 hours. A study team physician determined if the patients met criteria for discharge before patients were allowed to leave the treatment facility.

Outcomes

Outcomes were assessed at six time points for each patient (three per session; two sessions): at baseline (pretreatment), 2 hours after treatment for each session, and 24 hours after treatment for each session. A 1-week outcome assessment was not formally planned but was available as part of the baseline assessment for the second treatment session. The primary study endpoint was the change in the HDRS-21 at 24 hours after treatment. Secondary endpoints included change on the Quick Inventory of Depressive Symptomatology Self Report (QIDS-SR) scale. The primary mood assessment was selected to be administered at 24 hours to ensure that any acute euphoric effects of nitrous oxide had dissipated by this time (nitrous oxide euphoric effects typically cease shortly after discontinuation of nitrous oxide administration).

Psychiatric safety endpoints were assessed via careful clinical observations and questioning for dangerousness to self (suicidality) as well as for emergence of psychosis (hallucinations, delusions, disorganized thinking). Other safety endpoints included cardiovascular, respiratory, and central nervous system adverse events determined by hemodynamic and respiratory monitoring. The extent of nitrous oxide–induced inactivation of vitamin B_{12} was determined by measurement of plasma total homocysteine before and after treatment.

Statistical Analysis

The primary outcome (HDRS-21) was analyzed with a repeated-measures mixed effects linear model using restricted maximum likelihood estimation. To adjust for the observed carryover effect, the model included a randomization group term and a three-way interaction (treatment × time × randomization group). Also, we performed a similar repeated-measures mixed model for only the first treatment session (with a two-way interaction). These analyses were repeated for the QIDS-SR scale.

To compare the rates of treatment responses and remissions between the two treatments (using the paired data structure), an exact binomial test was used (and corresponding odds ratios [ORs] calculated) because the number of discordant pairs was <20. Data are presented as mean ± SD or 95% confidence intervals [CIs] or as median and

interquartile range.

Because this was the first in-human patient pilot study, no prior knowledge existed for adequate sample size determination. We based our sample size (20 patients with TRD) on available results from ketamine trials in similar populations, where a significant antidepressant effect was observed in <20 patients. JMP Pro 11.1 and SAS 9.3 (SAS Institute, Inc, Cary, North Carolina) and GraphPad Prism 6.04 (GraphPad Software, Inc, La Jolla, California) were used for the statistical analysis and graphing. All reported *p* values are two-sided, and a *p* value < .05 was considered statistically significant.

Results

Patients

Between November 2012 and February 2014, we enrolled 24 patients with TRD into the trial. After excluding three patients for screen failure, 21 patients were randomly assigned to a study group (Figure S1 in Supplement 1). One patient withdrew after the first session and before any outcomes could be assessed, leaving an evaluable patient population of 20 patients who received both treatments and completed the follow-up assessment. All results are reported from these 20 evaluable patients (modified intention-to-treat).

Patients had on average 19 lifetime years of major depressive disorder, failed a median of eight (adequate dose/duration) antidepressant drug treatments, and were taking a median of two antidepressants at the time of study participation (Table 1). The median HDRS-21 score at enrollment was 23.5 (interquartile range, 22.3–25.0), and the median QIDS-SR was 19 [interquartile range, 15.3–20.8], indicative of severe depression.

Table 1 Baseline Characteristics

Age, Years	48 (30–55)
Female Sex	12 (60%)
White Race	20 (100%)
Depression History, Years	19 (11–27)
Number of Failed Treatments	8 (4–12)
Baseline HDRS-21 Score	23.5 (22.3–25.0)
Baseline QIDS-SR Score	19 (15.3–20.8)
Vagus Nerve Stimulator	3 (15%)
History of ECT	4 (20%)
Number of Current Antidepressant Medications	2 (0–2)

Values are median and interquartile range or numbers and percentages.

ECT, electroconvulsive therapy; HDRS-21, 21-item Hamilton Depression Rating Scale; QIDS-SR, Quick Inventory of Depressive Symptomatology Self Report; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin–norepinephrine reuptake inhibitor.

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Treatment

The full 60-min treatment with nitrous oxide was completed by 15 patients; the treatment was interrupted for 5 min in 2 patients and was discontinued (at 55, 28, and 18 min for emotional discomfort, regurgitation, claustrophobia, or nausea and vomiting [Table 2]) in 3 patients. The mean duration of nitrous oxide treatment was 55.6 ± 2.5 (SD) min at an average inspiratory nitrous oxide concentration of 44% (37%–45%, median, interquartile range). All patients completed the full 60-min placebo treatment.

Table 2 Adverse Outcomes

Adverse Event	Nitrous Oxide	Placebo
Nausea and Vomiting	3 (15%)	0
Headache	2 (10%)	2 (10%)
Dizziness/Lightheadedness	1 (5%)	2 (10%)
Numbness/Paresthesia	2 (10%)	0
Anxiety	2 (10%)	0
Panic Attack	1 (5%)	0
Hypercapnia	0	1 (5%)
Claustrophobia	1 (5%)	0
Hyperventilation	1 (5%)	0
Regurgitation	1 (5%)	0

Values are numbers and percentages.

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Study Outcomes

Patients experienced a significant improvement in depressive symptoms at 2 hours and 24 hours after

receiving nitrous oxide (mean difference in HDRS-21 score at 2 hours, –4.8 points, 95% CI, –1.8 to –7.8 points, $p = .002$; at 24 hours, –5.5 points, 95% CI, –2.5 to –8.5 points, $p < .001$) compared with placebo (mean difference in HDRS-21 score at 2 hours, –2.3 points, 95% CI, .8 to –5.3 points, $p = .14$; at 24 hours, –2.8 points, 95% CI, .2 to –5.8 points, $p = .07$; comparison between nitrous oxide and placebo, $p < .001$) (Figure 1). Figure 2 shows the response within individual symptoms from the HDRS-21 that showed the biggest change: depressed mood, guilt, suicidal ideation, and psychic anxiety. On the QIDS-SR scale, patients experienced a significant reduction at 24 hours after nitrous oxide treatment (mean, –3.2 points, 95% CI, –1.3 to –5.0 points, $p = .001$ between baseline and 24 hours) compared with placebo (mean, –1.0, 95% CI, .9 to –2.8 points, $p = .32$; comparison nitrous oxide vs. placebo, $p = .003$) (Figure S2 in Supplement 1).

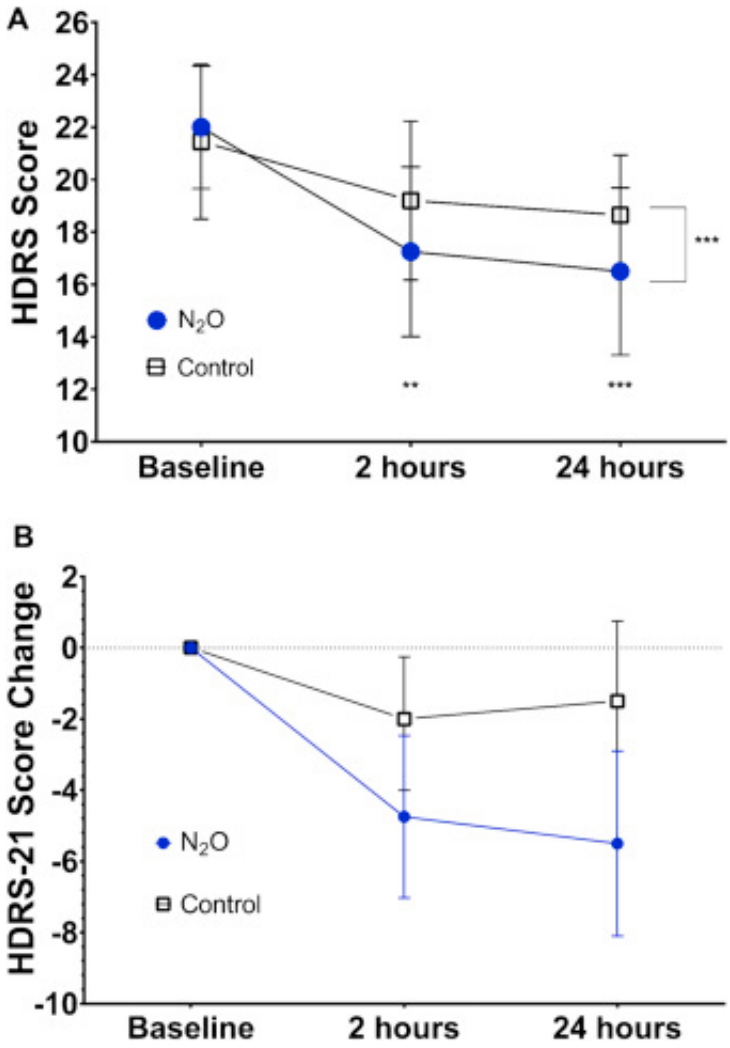

Figure 1 Effects of nitrous oxide treatment on depressive symptoms measured on the 21-item Hamilton Depression Rating Scale (HDRS-21). (A) Absolute change on the HDRS-21. (B) Normalized response (adjusted for baseline) on the HDRS-21. Patients were evaluated at three time points: baseline (pretreatment), 2 hours after treatment completion, and 24 hours after treatment completion. Nitrous oxide provided a significantly more pronounced reduction in depressive symptoms compared with placebo ($p < .001$). Data are presented as mean ± 95% confidence interval. Blue circles, nitrous oxide (N_2O); squares, control (placebo); **$p < .01$; ***$p <$

.001.

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Figure thumbnail gr2

Figure 2 Individual depressive symptoms from the 21-item Hamilton Depression Rating Scale. The four depressive symptoms on the 21-item Hamilton Depression Rating Scale that showed the largest change (depressed mood, guilt, suicidal ideation, and psychic anxiety) are depicted as color-coded bar graphs in order of severity (red = severe, white = absent) between the six different time points of the trial. BL, baseline; N_2O, nitrous oxide.

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At 24 hours, four patients (20%) had treatment response (defined as reduction in depressive symptoms ≥50% on the HDRS-21) after receiving nitrous oxide compared with one patient (5%) after placebo treatment (OR, 4.0, 95% CI, .45–35.79) (Figure 3A). Three patients (15%) had a full remission after nitrous oxide treatment (defined as complete resolution of depressive symptoms, HDRS-21 ≤ 7 points); no patients had full remission after placebo (OR, 3.0, 95% CI, .31–28.8) (Figure 3B).

Figure thumbnail gr3

Figure 3 Clinical outcomes after nitrous oxide and placebo treatment. Rates of response (A) (defined as a reduction in 21-item Hamilton Depression Rating Scale score ≥50%) and remission (B) (defined as complete resolution of depressive symptoms, 21-item Hamilton Depression Rating Scale score ≤7) 24 hours after treatment are shown. Compared with placebo, nitrous oxide had a fourfold higher response (odds ratio, 4.0, 95% confidence interval, .45–35.79) and threefold higher remission rate (odds ratio, 3.0, 95% confidence interval, .31–28.8).

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Subdividing the HDRS-21 scale into five levels of depression severity (normal, mild, moderate, severe, very severe), 7 of 20 patients (35%) had at least a two-level improvement 24 hours after receiving nitrous oxide (i.e., from severe to mild) compared with 2 patients receiving placebo (10%; $p = .06$) (Table 3). Table S1 in Supplement 1 shows the response on the QIDS-SR scale.

Table 3 Change in Level of Depression Severity 24 Hours After Treatment

	Nitrous Oxide		Control	
Worse	↑ 1	0	↑ 1	1/20 (5%)
Neutral	0	6/20 (30%)	0	12/20 (60%)
Better	↓ 1	7/20 (35%)	↓ 1	5/20 (25%)
	↓ 2	3/20 (15%)	↓ 2	1/20 (5%)
	↓ 3	3/20 (15%)	↓ 3	1/20 (5%)
	↓ 4	1/20 (5%)	↓ 4	0

Relative change after treatment according to the five levels of depression severity on the 21-item Hamilton Depression Rating Scale (normal, mild, moderate, severe, very severe). Downward arrows indicate improvement; upward arrows indicate worsening of depressive symptoms compared with baseline. For example, a two-level improvement would be from severe depressive symptoms to mild.

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First Treatment Session–Only Analysis

In this crossover trial, we expected depressive symptoms to revert to baseline after 1 week when patients returned for their second treatment session.

However, several patients showed markedly lower HDRS-21 scores after the 1-week interval, indicating a significant carryover effect ($p = .02$). The heat map in Figure 4 shows a significant difference between HDRS-21 scores of the 10 patients who received nitrous oxide first and the 10 patients who received placebo ($p = .02$ for difference between randomization groups). To address this carryover effect, we additionally analyzed the first treatment session only (i.e., compared the 10 patients who received nitrous oxide with 10 who received placebo, akin to a parallel group design). This analysis allowed us to include 1-week outcomes because it represents the baseline assessment for the second treatment session.

Figure thumbnail gr4

Figure 4 Cell plot (heat map) of individual responses of first treatment session. Nitrous oxide ($n = 10$) on the left and placebo ($n = 10$) on the right, measured on the Hamilton Depression Rating Scale colored to indicate severity of symptoms (red = severe, blue = less severe). Each row represents an individual patient. Patients in the left plot are different from the ones in the right plot. HDRS, Hamilton Depression Rating Scale.

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Patients who received nitrous oxide first (n = 10) had a significant improvement of depressive symptoms at 2 hours, 24 hours, and 1 week (mean reduction of HDRS-21 at 2 hours, –7.1 points, 95% CI, –2.4 to –11.8 points; at 24 hours, –8.6 points, 95% CI, –4.4 to –12.8 points; at 1 week, –5.5 points, 95% CI, –.8 to –10.2 points) compared with patients who received placebo first (n = 10) (at 2 hours, –2.9 points, 95% CI, 1.7 to –7.6 points; at 24 hours, –4.7 points, 95% CI, –.0 to –9.4 points; at 1 week, –4.4 points, 95% CI, .3 to –9.1 points) (Figure 5).

Figure thumbnail gr5

Figure 5 Effects of nitrous oxide treatment on depressive symptoms for only the first treatment session (10 patients each) measured on the 21-item Hamilton Depression Rating Scale (HDRS-21). Absolute (A) and relative (B) changes on the HDRS-21 compared with baseline (pretreatment) and 2 hours, 24 hours, and 1 week after treatment. Nitrous oxide provides a significantly stronger reduction in depressive symptoms compared with placebo. The HDRS-21 scores at 1 week were derived when patients returned for their second session (baseline HDRS-21 score for session 2). The HDRS-21 scores 1 week after nitrous oxide treatment are significantly lower than at baseline, indicative of a sustained treatment effect. N_2O, nitrous oxide.

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Safety

No serious adverse events occurred. All adverse events (Table 2) were temporary. No increase in plasma total homocysteine was observed after nitrous oxide or placebo treatment, indicating minimal inactivation of vitamin B_{12}–dependent metabolism by nitrous oxide (Figure S3 in Supplement 1).

Discussion

This proof-of-concept trial demonstrated that nitrous oxide has rapid antidepressant effects in patients with TRD. These antidepressant effects were sustained for at least 24 hours and in some patients for 1 week. Nitrous oxide resulted in a treatment response in 20% of patients with TRD and remission in 15%. Although a subset of patients experienced adverse events requiring a short interruption or discontinuation of treatment, the mild to moderate nature and immediate reversibility of these events (nausea, anxiety, vomiting) suggest an acceptable risk/benefit ratio for nitrous oxide use in the setting of TRD.

The internal validity of our crossover trial was affected by the observed carryover effect (i.e., patients having a different baseline at different treatment sessions). In our study, several patients

who returned for their second treatment session had markedly lower depression scores. Typically, carryover effects bias results toward the null hypothesis (i.e., reduce the observable effect size) (29, 30). This was the case in our study: the 10 patients who received nitrous oxide treatment first had a mean reduction in depressive symptoms of 8.6 points on the HDRS-21 compared with 5.5 points for the full cohort. This observation supports the notion that nitrous oxide has true antidepressant efficacy. A second effect that influenced the internal validity of our trial was the presence of a placebo effect. Placebo effects are common in trials of antidepressants (22, 31, 32) and may introduce bias by masking or exaggerating treatment effects.

Pilot studies, such as this early phase II clinical trial, are designed to detect an efficacy signal in a small group of patients and cannot provide robust and definitive measures of effectiveness. Pilot trials should be interpreted with caution because results must be replicated in larger cohorts. Although the antidepressant efficacy results in this trial are promising, several potential limitations should be taken into consideration. First, although our study team went to great lengths to maintain blinding, the euphoric effects of nitrous oxide inhalation are difficult to mask. Nitrous oxide induces sedation and has a slightly sweet smell and taste. It is possible that some patients were able to determine whether they were receiving nitrous oxide or placebo inhalation. We did not test patients to determine if

they were aware of their group assignment, and this limits our conclusions. We intentionally selected the 24-hour postinhalation mark as the primary measure to minimize acute euphoric effects. However, there remains the possibility that nitrous oxide inhalation may have produced a “masking” of depressive symptoms (i.e., the depressive symptoms were not really altered, but rather “covered up” by other effects). Symptom “masking” has been observed with rapidly acting psychostimulants (methylphenidate and cocaine), which promote a transient alteration in mood but not a true antidepressant effect (33, 34).

Second, although we clinically assessed the presence of euphoria and psychosis at each time point, we did not do standardized testing of either. In general, at 2 hours and 24 hours, the patients did not report euphoric feelings. Third, the use of the HDRS-21 and QIDS-SR scales to measure rapid antidepressant action was a limitation because both scales assess symptom changes occurring over the course of days and weeks rather than hours, including questions related to sleep and weight, and are not ideal for assessing changes in antidepressant action that occur rapidly. Different scales, such as the International Positive and Negative Affect Schedule Short Form or a visual analog scale, might have been superior. Fourth, we had no prior knowledge about dosing in this patient population and opted to use a 50% inspiratory concentration of nitrous oxide, a dose commonly used in dentistry and obstetric analgesia.

Subsequent studies may determine that different dosing regimens improve efficacy and tolerance.

Compared with ketamine, the most commonly investigated NMDA receptor antagonist drug in major depressive disorder, nitrous oxide had a similarly rapid onset of antidepressant action (within 2 hours) but appeared to be devoid of psychotomimetic side effects seen with ketamine (delusions, illusions, hallucinations), which may result from the more favorable pharmacokinetics of nitrous oxide because its offset occurs on the order of minutes (22, 27, 35, 36, 37). The fact that both ketamine and nitrous oxide have antidepressant effects in patients with TRD supports the notion that NMDA receptor signaling plays a crucial role in the neurobiology of major depressive disorder (18, 19, 20, 21, 38, 39). However, recent data indicate that other neurotransmitter receptor systems, including nicotinic acetylcholine receptors, may be important contributors to rapid antidepressant actions (40, 41).

We can only speculate why certain NMDA receptor antagonists (ketamine, nitrous oxide) appear to have rapid antidepressant properties, whereas others, such as memantine, do not. Differences in NMDA receptor channel blocking seem unlikely to contribute because differences between ketamine and memantine are often observable only under extreme depolarization or pathologic receptor activation (simulated ischemia) (42). The presence of extracellular magnesium may distinguish the effects

of ketamine and memantine on NMDA receptors, with memantine being relatively ineffective against NMDA receptor–mediated synaptic currents in magnesium (43). This latter effect also appears to contribute to differences in the ability of the two drugs to promote brain-derived neurotrophic factor production. Differences in mode of administration and pharmacokinetics may also contribute to observed clinical differences between ketamine and memantine. Although nitrous oxide, similar to ketamine, is a noncompetitive NMDA receptor antagonist, it differs from ketamine in lacking use dependence and is not a trapping open channel blocker (15). Nitrous oxide represents an alternative way to modulate NMDA receptor function clinically.

Although a single administration of 50% nitrous oxide/oxygen has been found to be generally safe (4% nonserious adverse event rate among 25,828 patients receiving sedation (44)), two potential safety concerns exist. First, nitrous oxide administration had to be interrupted or discontinued in a subset of our patients (typically near the end of the 1-hour treatment session), and the adverse event profile indicates that some patients may experience emotional discomfort, paradoxically increased anxiety levels, and nausea during nitrous oxide administration. Although nearly all side effects were limited to the immediate treatment period and disappeared shortly after discontinuation, their nature suggests that perhaps a shorter treatment duration or lower nitrous oxide concentration may be

advantageous.

A second potential safety concern relates to inactivation of vitamin B_{12} by nitrous oxide (45, 46). Although a single exposure is unlikely to result in clinically relevant hematologic or neurologic complications (47, 48), the risk for such complications is substantially higher when nitrous oxide administrations are repeated within short periods of time (5). Hematologic and neurologic complications, such as megaloblastic anemia and myelopathy, have been reported among persons who chronically abuse nitrous oxide (49) and patients with chronic disturbances of folate metabolism (50, 51). It is likely that for sustained antidepressant effect, nitrous oxide must be administered several times, which would increase the risk for such complications. Nitrous oxide is a drug of abuse, and its abuse potential represents a potential limitation for its clinical utility in major depressive disorder. Our pilot study was not designed to address this safety concern.

In conclusion, this preliminary, proof-of-concept clinical trial provides the first evidence that nitrous oxide may have rapid and marked antidepressant effects in patients with TRD. Subsequent studies are required to determine optimal antidepressant dosing strategies and the risk/benefit ratio of nitrous oxide in a larger and more diverse population of patients with TRD.

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PN has filed for intellectual property protection related to the use of nitrous oxide in major depression and has received research support from Roche Diagnostics, Abbot, and Express Scripts

unrelated to this work. CFZ serves on the Scientific Advisory Board of Sage Therapeutics; Sage Therapeutics was not involved in this study. CC was previously on the speaker's bureau for Bristol-Myers Squibb and Otsuka Pharmaceuticals and has received research funding from Bristol-Myers Squibb, Cyberonics, the Stanley Baer Foundation, and the Brain and Behavior Research Foundation. All other authors report no biomedical financial interests or potential conflicts of interest.

ClinicalTrials.gov: Nitrous Oxide as Treatment for Major Depression—a Pilot Study; http://clinicaltrials.gov/show/NCT02139540.

Appendix A. Supplementary materials

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Supplementary Material

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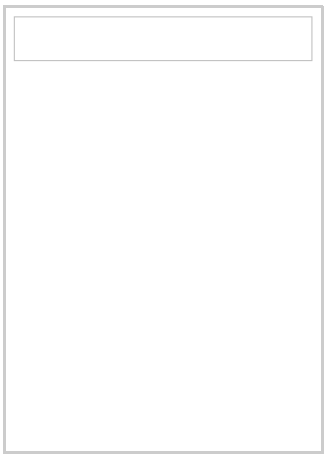

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Figure 2 Individ…

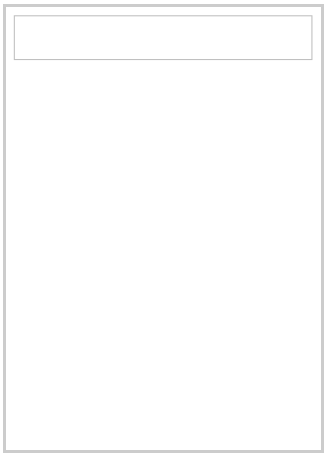

Figure 3 Clinica…

Figure 4 Cell pl…

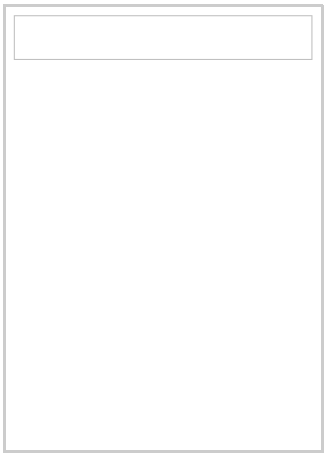

Figure 5 Effects…

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