A Randomized, Double-blind, Placebo-controlled Clinical Trial to Evaluate the Safety and Tolerability of Molecular Hydrogen in Patients with Parkinson's Disease

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INVESTIGATORS

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A. OBJECTIVES

Primary Objective: To investigate the safety and tolerability of molecular hydrogen H_2 in patients with Parkinson's disease (PD).

Secondary Objective: To investigate the efficacy of molecular hydrogen H_2 in slowing the progression of symptoms in patients with PD.

B. BACKGROUND AND SIGNIFICANCE

PD is an increasingly prevalent neurodegenerative disorder. Current predictions estimate that the number of individuals with PD will double by 2040, increasing from 6.9 million in 2015 to 14.2 million in 2040[1]. Disease-modifying therapy that slows the rate of progression of neurodegeneration remains one of the greatest unmet therapeutic needs in PD.

PD is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Several lines of evidence suggest that oxidative stress plays a critical role in this process. Oxidative stress occurs when endogenous antioxidant systems become overwhelmed by the generation of reactive oxygen species (ROS), which occurs primarily at the level of the mitochondria. Mitochondrial dysfunction is recognized as being critical to PD pathogenesis, with several of the genes associated with recessively inherited PD, including *parkin*, *PINK1* and *Fbxo7*, recognized as key players in the mitophagy of damaged mitochondria. Post-mortem studies have also provided strong evidence for oxidative damage in PD brains. Lipid peroxidation, the degradation of cell membrane components mediated by ROS, has been demonstrated to be increased in the SN of PD patients[2]. In addition, an altered ratio of reduced to oxidized glutathione (GSH/GSSG) has been detected in the SN of PD patients, consistent with a role for oxidative stress in the pathogenesis of nigral cell death.[3]

Given the substantial evidence suggesting that oxidative stress may contribute to PD pathogenesis, antioxidant molecules have long been considered attractive as potential disease-modifying agents. Clinical trials examining the role of antioxidants such as vitamin E, coenzyme Q_{10}[4], and creatine[5] as disease-modifying agents in PD have unfortunately failed to demonstrate efficacy of these agents in slowing disease progression. The failure of these agents, however, may have been secondary to significant limitations in antioxidant efficacy and access to cellular compartments where highly reactive, pathogenic radicals are generated. Despite these failures, targeting oxidative stress in PD remains a promising line of investigation. Recent data have in fact identified an inverse relationship between levels of the antioxidant urate and both PD risk[6]

and rate of disease progression[7, 8]. Given these promising epidemiological findings, a phase 2 study demonstrating safety and tolerability of the urate precursor inosine has now been completed[9], and a phase 3 trial testing whether inosine slows clinical decline in PD patients is currently underway.

H_2 is another recently emergent and potentially therapeutic antioxidant. H_2 acts as a powerful yet selective antioxidant, efficiently penetrating bio-membranes to quench toxic free radicals including •OH and $ONOO^-$ while having little effect on free radicals with important roles in normal cellular signaling such as O_2^-, NO, and H_2O_2[10]. Work in PD rodent models using the neurotoxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) have demonstrated that H_2-enriched water acts as both a highly effective antioxidant as well as a cytoprotective agent, downregulating markers of oxidative stress in dopaminergic neurons, attenuating dopaminergic neuronal loss, and improving parkinsonian features[11, 12].

We propose to perform a clinical study to investigate the safety and tolerability of molecular hydrogen, a promising antioxidant agent, in PD. We intend to explore the efficacy of this agent in slowing disease progression as a secondary objective, with the goal of determining whether and how H_2 should be pursued in subsequent phase 3 clinical trials.

C. PRELIMINARY STUDIES

A recent small pilot randomized double-blind placebo-controlled trial involving a total of 17 patients with idiopathic PD treated with levodopa (Hoehn & Yahr stages I – IV; average disease duration of 6.5 and 7.2 years for active treatment and placebo arms, respectively) demonstrated that drinking 1000 mL/day of H_2-enriched water for a total of 48 weeks was safe and well-tolerated in this patient cohort, with no adverse events reported. Notably, patients in the active treatment arm demonstrated significant improvement in total scores on the Unified Parkinson's Disease Rating Scale (UPDRS) when compared to placebo (-5.7 ± 8.4 vs. 4.1 ± 9.2 for active treatment vs. placebo, respectively). Changes in the motor subscale of the UPDRS (UPDRS-III), a secondary outcome measure, trended towards significance (-5.8 ± 7.2 vs. 2.3 ± 8.5 for active treatment vs. placebo, respectively; $p = 0.074$)[13]. Despite the promising results from this small pilot study, its design is inherently unable to differentiate between disease-modifying and symptomatic effect. Additionally, the recruited patients were relatively advanced in the course of their disease, when the pool of existing dopaminergic neurons was likely quite low, and the disease-modifying potential for the treatment therefore diminished.

A follow-up randomized double-blind placebo-controlled multi-center trial to assess the efficacy of H_2 in a larger sample of PD patients, including those not on levodopa, is currently underway.[14] Several aspects of the design of this ongoing study are concerning, however. Enrolled patients had an average disease duration of 6.8 ± 4.5 years at time of study onset, indicating that many of the study participants have a small pool of salvageable dopaminergic neurons. In addition, the H_2-enriched water provided to patients in this study is distributed weekly in plastic bottles, through which H_2 is able to rapidly diffuse over a period of minutes to hours. The H_2 concentration at the time of consumption is thus unclear.

Here, we propose a double-blind placebo-controlled study to investigate the safety and tolerability, and to explore the efficacy, of H_2-enriched water in a population of early-stage PD

patients. We seek to enroll a total of 70 patients with early-stage PD, and will make use of a practical method our department has considerable experience with in order to generate H_2-enriched water.

D. RESEARCH DESIGN AND METHODS

D1. Rationale/Overview

The therapeutic potential of H_2 stems from both its safety and ease of administration in humans as well as its selective mechanism of action.

Importantly, exposure to H_2 is not associated with any known biological toxicity. Molecular hydrogen occurs naturally as a trace gas in air at a concentration of about 0.5 ppm, and can reach a concentration of 7500 ppm (0.75%) in closed environments such as aboard nuclear submarines. In 2008, a National Academies study group exploring the long-term effects of hydrogen inhalation in submariners concluded that hydrogen did not have known toxic activity except at concentrations high enough to replace ambient oxygen. The group recommended setting occupational exposure standards based upon consideration of explosivity, noting that hydrogen becomes flammable in air at a concentration of 4.1% (Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants, 2008). H_2 can be safely administered in numerous ways: inhaling H_2 gas, drinking H_2-enriched drinking water, injecting H_2-dissolved saline, as well as using H_2-enriched eye drop solution[15].

H_2 readily diffuses through tissues and cell membranes, and acts as a powerful yet selective antioxidant[10]. Cytoprotective effects have been described after H_2 administration in various rodent models of disease, including ischemic hepatic injury[16], ischemic myocardial infarction[17], and radiation-induced lung damage[18]. In mice subjected to chronic physical restraint stress, continuous ad libitum consumption of H_2-enriched water suppressed the increase in oxidative stress markers and prevented the stress-induced decline in learning and memory induced by chronic restraint[19]. In rodent models of PD, ad libitum consumption of H_2-enriched water downregulated markers of oxidative stress in dopaminergic neurons, attenuated dopaminergic neuronal loss, and improved parkinsonian features[11, 12] While the primary target underlying cytoprotective effects in these models remains unclear, several possible mechanisms have been suggested, including direct scavenging of ROS as well as indirect effects on gene expression impacting downstream inflammatory and apoptotic factors[15].

In addition to these animal studies, several dozen clinical studies exploring the efficacy in H_2 in treating human diseases ranging from rheumatoid arthritis to metabolic syndrome have been reported[20]. Randomized clinical trials in neurodegenerative conditions such as mild cognitive impairment in APOE4 carriers[21] and PD[13] have reported promising findings that merit further validation in larger, randomized, placebo-controlled clinical trials.

D2. Research Site

Eligible participants will be recruited at the Stony Brook Movement Disorders Center, where several newly diagnosed PD patients are typically seen each week. The offices of the investigators and study coordinator are located at the Stony Brook University Health Sciences Center. The subjects will be seen at the Stony Brook Neurology Clinic or research sites affiliated with Stony Brook University.

Stony Brook is the only academic medical center in Suffolk County, a large area with a population of 1.5 million people. We expect to be able to draw subjects from our region, including other nearby population centers in NY and CT. We intend to recruit non-Stony Brook subjects through the posting of the study on the website www.clinicaltrials.gov.

D3. Study Sample

Subjects with idiopathic PD who meet the inclusion/exclusion criteria in Table 1 below and provide consent will be recruited and randomized sequentially 1:1 to either receive H_2 or placebo.

We seek to enroll 70 eligible subjects diagnosed with idiopathic PD, as per UK Parkinson's Disease Society Brain Bank criteria[22]. We project an active recruitment period of 3 years.

D4. Study design

This will be a double-blinded, placebo-controlled trial. Subjects will be sequentially randomized in a 1:1 ratio to receive H_2-enriched water or the corresponding placebo, in addition to all standard-of-care treatments. Enrollment goal is 70 subjects.

Subjects can withdraw from the study at any time.

D5. Study procedures

D5. a. Recruitment

Investigators will review the medical records of their patients who are due for a visit at the Stony Brook Neurological Associates to identify eligible patients. Study coordinators will contact these patients before their visit to ascertain interest in participating in a clinical trial. If so, the study coordinator will send the patient the consent forms (via mail/fax/email), and review the forms with the patient over the phone. The patient will also have the opportunity to speak with Dr. Maurer or one of the co-investigators about the consent form at their visit. For potential study subjects who are under the care of another neurologist, the treating physician will first approach the patient to obtain the patient's verbal consent for study staff to contact them about the study.

Inclusion/exclusion criteria are listed in Table 1.

D5. b. Screening visit

A study staff member will complete the inclusion/exclusion checklist (Table 1) based on review of the patient's medical records. The PI or co-investigator will review and sign the completed inclusion/exclusion checklist(s) after the patient is seen at the screening visit.

Table 1: Inclusion/exclusion criteria

Inclusion criteria	• Age 40 - 80 • Diagnosis of idiopathic PD with bradykinesia plus one of the other cardinal signs (resting tremor, rigidity), as confirmed by a movement disorder specialist according to UK Parkinson's Disease Society Brain Bank criteria[22] • Modified Hoehn & Yahr Stage < III • Diagnosis of PD made within past 3 years • Ability to provide informed consent • Strong enough familiarity with the English language to undergo careful assessment of cognition • Ability to complete questionnaires • Willingness to go off parkinsonian medication for 12 hours prior to baseline and 56-week assessments
Exclusion criteria	• Other major diseases of the central nervous system, including brain tumors, demyelinating disease, epilepsy, Alzheimer's disease, inflammatory brain or vascular disease, traumatic encephalopathy • History of stroke • Use of antipsychotic neuroleptic medication within the last 6 months • Symptomatic (secondary) parkinsonism (ie. drug induced) • Atypical parkinsonian variants (ie. progressive supranuclear palsy, multi-system atrophy, corticobasal syndrome) • Unstable medical or psychiatric illness that may compromise participation in the study • Known kidney disease • History of stereotactic brain surgery • Significant cognitive impairment • Inability to safely tolerate 500 mL fluid PO water daily associated with the study medication • Unable to avoid regular use of medications containing magnesium • Treatment with another investigational drug within the last 30 days that may interfere with the study medication • Inability to tolerate or comply with study procedures • Pregnancy or nursing. Females of childbearing potential* must have a negative urine pregnancy test result at screening, and use an effective method of contraception throughout the entire study period and for 30 days after study drug discontinuation.

* All females will be considered to be of childbearing potential unless they are postmenopausal (amenorrheic for at least 12 consecutive months, in the appropriate age group, and without other known or suspected cause of amenorrhea) or have been sterilized surgically (i.e., bilateral tubal ligation, total hysterectomy, or bilateral oophorectomy).

At the screening visit, the study staff will review the consent form with the patient, including the purpose of the study, the procedures to be followed, and the risks and benefits of participation. As part of the informed consent process, the subject will be counselled specifically about side effects to look out for, and will be told to contact the PI immediately to report the occurrence of side effects. The study doctor will meet with the patient to discuss any concerns or questions about the study. If the patient would like more time to make a decision, the baseline visit will be postponed until patient agrees to participate and signs the consent form. After the consent form has been signed, the patient will be given a copy of the signed consent form.

Pregnancy test (as needed) will also be performed at the screening visit.

D5. c. Baseline visit

The baseline visit will occur no more than 4 weeks after the screening visit, and may occur on the same day as the screening visit if the patient is not taking dopaminergic medication.

The following data will be collected at the baseline visit.

Table 2. Baseline data

Data	Detail
General health status	Physical examination findings Neurological examination findings Concomitant medications (including review of use of over the counter and prescription medications containing magnesium) Height, weight, orthostatic vital signs*
PD symptom burden	Total Unified Parkinson's Disease Rating Scale (MDS-UPDRS) score (parts I – IV)
Health-related quality of life	Parkinson's Disease Questionnaire-39 (PDQ-39)
PD stage	Modified Hoehn & Yahr staging
Cognitive status	Montreal Cognitive Assessment (MoCA)
Emotional status	Geriatric Depression Scale – short form (GDS-15)
Uric acid	Blood draw

If subjects are taking parkinsonian medication, we will ask them to be off this medication for at least 12 hours prior to their baseline visit in order to perform assessment of their motor symptoms (MDS-UPDRS part III) and PD stage (Modified Hoehn & Yahr) in the practically defined "off" medication state. This is a standard procedure for this type of study[23]. Subjects must be willing to undergo this washout procedure to participate in the study. Assessment of cognitive status will also be performed in the practically defined "off" medication state. After motor assessment and PD staging is performed in the "off" state, patient will be instructed to take his/her parkinsonian medication in the office. The motor subscale (MDS-UPDRS part III) will then be performed, and PD stage (Modified Hoehn & Yahr) will be assessed, in the best

"on" state (best response to morning dose of antiparkinsonian drug) 30-60 minutes after medication has been taken.

* Orthostatic vital signs will be obtained by measuring vital signs after 5 minutes supine and after 2 minutes standing. We will obtain 3 sets of orthostatic vital signs at least 15-20 minutes apart. The average of the 3 orthostatic vital signs measurements will be used for comparison to all post-treatment measurements.

Subjects will be instructed to avoid the regular use of over the counter antacids, laxatives, and cathartics that contain magnesium while taking study medication.

Serum uric acid level will be drawn at the baseline visit given evidence suggesting that there may be an inverse relationship between levels of uric acid and rate of PD progression[7, 8]. Uric acid level is for research purposes only.

The PI or co-investigator will once again review the inclusion/exclusion checklist at the time of the baseline visit.

D5. d. Treatment assignment & administration

For subjects meeting enrollment criteria and providing consent, treatment with H_2-enriched water or placebo will begin after the baseline visit. The subject will be randomly assigned to either the active treatment or the placebo group. A blocked randomization list will be generated using the following website: https://www.sealedenvelope.com/simple-randomiser/v1/lists. The research pharmacist will be responsible for using the generated list to assign treatment to subjects as they are enrolled. The study staff assessing study outcomes will remain blinded to treatment assignment. The study doctor will dispense study drug to the subject. Masking of active and placebo treatment will be maintained by bottling tablets in containers that appear identical.

The medication will be administered orally as 250 mL BID of H_2-enriched or placebo drinking water. Subjects will be instructed to adhere to a q6h – q10h schedule, but for convenience a dose may be taken a few hours earlier or later, as there is no expected mechanism of action indicating that dose timing would be critical for efficacy.

D5. e. Follow-up visits

Follow-up visits will be scheduled to occur at weeks 8, 24, 40, 52, and 56 (± 4 weeks). Adverse effects will be monitored to assess safety and tolerability in this patient population. The study doctor will dispense study drug to patient at weeks 8, 24, and 40. The subject will be counseled to call the investigator immediately to report the occurrence of any adverse events. Subjects will be instructed to return bottles with unused tablets at each follow-up visit. Tablet counts will be performed by the research pharmacist to monitor patient compliance.

As a temporary measure to allow for continuity of this study during the COVID-19 pandemic, follow-up visits will be conducted remotely via telephone and/or secure videoconferencing using

the Microsoft Teams application. The following modifications will be made to follow-up visits during this time period:

1. The study medication will be mailed to the subject instead of given to them in person. The study coordinator will pick up the medication from the research pharmacy at least 4 days prior to the follow-up visit and bring it to the Stony Brook Post Office and have it shipped via Priority Mail. The study coordinator will email the subject the day that the medication goes out by mail to let them know to look for it and will request that the subject email or call to confirm once they have received it. The study coordinator will call two days after the medication has been shipped to confirm it was received if the subject has not called by then.
2. Instead of bringing the old container of study medication to the follow-up visit, subjects will be instructed to count the number of tablets in the container the morning of their follow-up visit (after taking the morning dose for that day) and reporting that number to the researcher during their follow-up visit. They will also be instructed to bring the container to their next in-person visit.
3. Patients will not receive a physical examination or neurological examination. While these examinations do provide some meaningful information, they are not necessary for the primary and secondary outcomes of the study.
4. A modified version of the motor portion of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS III) will be performed remotely with the exception of rigidity testing and postural instability testing which both require hands-on assessment by the rater. The modified UPDRS (mUPDRS) has demonstrated evidence of validity and reliability.[24]
5. A modified version of the Montreal Cognitive Assessment (MoCA) will be administered. Performing the MoCA remotely via telemedicine has been shown to be feasible in movement disorders populations.[25]
6. Survey based assessments (MDS-UPDRS I, II, IV, GDS-15, and PDQ-39) will be administered online via RedCap.
7. Height and weight will be measured by the subject, if they have the ability to do so, and reported during their visit. A notation will be added to the dataset indicating that these were measured by the subject since these measures may not be consistent with those that would have been obtained at the clinical research center.
8. Instructions for measuring orthostatic vital signs will be provided to subjects prior to the follow-up visit. If they have a means for measuring blood pressure they will take these measurements and report them at their visit. A notation will be added to the dataset indicating that these were measured by the subject since these measures may not be consistent with those that would have been obtained at the clinical research center.
9. The research coordinator will call subjects weekly to inquire about adverse events beginning the week after the remote visit has taken place as a safeguard given the inability to conduct a physical and neurological exam and to have the research nurse measure orthostatic vital signs. Weekly calls will continue until the next in-person visit has taken place.
10. If restrictions due to COVID-19 are lifted within 4 weeks of the remote visit, an in-person visit will be scheduled and the physical examination, neurological examination, MDS-UPDRS III, Hoehn and Yahr Staging, and orthostatic vital signs will be taken during this visit.

11. Subjects will be sent a new consent form and have the changes reviewed by phone. They will be asked to sign the consent form and return via email or paper mail. Once received, the form will be signed by the study team member obtaining consent and sent back to the subject.

The following data will be collected at follow-up visits at weeks 8, 24, 40 and 52.

Table 3. Follow-up data at weeks 8, 24, 40 and 52

Data	Detail
General health status	Physical examination findings[a] Neurological examination findings[a] Concomitant medications Height, weight, orthostatic vital signs[b]
PD motor symptoms	MDS-UPDRS part III[c]

The following modifications will be made for visits taking place remotely, as will be required during the COVID-19 pandemic:

[a]These measurements will not take place for visits taking place remotely

[b]These measures will be taken by the subject, if they have the capacity to do so, for visits taking place remotely

[c]A modified version of this measure will be administered for visits taking place remotely

Orthostatic vital signs will be obtained by measuring vital signs after 5 minutes supine and after 2 minutes standing.

The following data will be collected at the follow-up visit at week 56.

Table 4. Follow-up data at week 56

Data	Detail
General health status	Physical examination findings[a] Neurological examination findings[a] Concomitant medications Height, weight, orthostatic vital signs[b]
PD symptom burden	MDS-UPDRS score (parts I, II[d], III[c], IV)
Health-related quality of life	Parkinson's Disease Questionnaire-39 (PDQ-39)[d]
PD stage	Modified Hoehn & Yahr staging
Cognitive status	Montreal Cognitive Assessment (MoCA)[c]
Emotional status	Geriatric Depression Scale – short form (GDS-15)[d]

The following modifications will be made for visits taking place remotely, as will be required during the COVID-19 pandemic:

[a]These measurements will not take place for visits taking place remotely

[b]These measures will be taken by the subject, if they have the capacity to do so, for visits taking place remotely

[c]A modified version of this measure (mUPDRS) will be administered for visits taking place remotely

[d]These measures will be administered online via RedCap for visits taking place remotely

In a similar manner to the baseline visit, if subjects are on parkinsonian medication, assessment of motor symptoms (UPDRS-part III) and PD stage (Modified Hoehn & Yahr) at week 56 will

be performed in the practically defined "off" and "on" medication states. Assessment of cognitive status will be performed in the practically defined "off" medication state.

Orthostatic vital signs will be obtained by measuring vital signs after 5 minutes supine and after 2 minutes standing.

D5. f. Follow-up phone calls

After the screening/baseline visit, the study coordinator will follow-up with the patient by phone at weeks 4, 16, 32, and 48. During the phone calls, the study coordinator will ask the subject about any adverse effects from the study drug. The subject will be reminded to call the investigator to report any adverse events. Subjects will also be asked if they have been compliant with taking the study medication as instructed.

After the last visit, the study coordinator will follow-up with the subject one last time by phone. The purpose of this phone call will be to ascertain the subject's medical status, to determine whether any ongoing adverse events present at the last clinic visit have resolved, and to inquire if any other adverse events have occurred since the last visit.

D5. g. Early Termination

Subjects will have the right to voluntarily discontinue study treatment and study visits at any time and for any reason.

Occurrence of severe adverse events attributed to study participation will result in immediate withdrawal from the study and termination of study medication. Subjects who are not compliant with taking the study medication as instructed will also be withdrawn early from the study.

Subjects who discontinue the study drug prior to week 52 will be seen for a final visit as soon as possible after study drug discontinuation.

Table 5. Study Overview

Assessments	Screening visit	Baseline visit (Week 0)	Phone contact (Weeks 4, 16, 32, 48)	Follow-up (Weeks 8, 24, 40, 52)	Final visit/ Early termination (Week 56)	Phone contact (Week 58)
Informed Consent	x					
Inclusion/Exclusion Criteria	x					

Medical history		x		x	x	
Physical examination		x		x[a]	x[a]	
Neurological examination		x		x[a]	x[a]	
Height, weight, orthostatic vital signs		x		x[b]	x[b]	
MDS-UPDRS, parts I, II, IV		x			X[c]	
MDS-UPDRS, part III (obtained in "off" and "on" states)		x			X[d]	
MDS-UPDRS, part III (obtained in "on" state)				X[d]		
Modified Hoehn & Yahr (obtained in "off" and "on" states)		x			X	
PDQ-39		x			X[c]	
Pregnancy test (if needed)	x					
GDS-15		x			X[c]	
MoCA (obtained in "off" state)		x			X[d]	
Adverse event review			x	x	x	x
Randomization		x				
Record concomitant medications		x	x	x	x	x
Study drug dispensation		X		x[e]		
Tablet count				X		

Blood draw		X				

The following modifications will be made for visits taking place remotely, as will be required during the COVID-19 pandemic:
[a]These measurements will not take place for visits taking place remotely
[b]These measures will be taken by the subject, if they have the capacity to do so, for visits taking place remotely
[c]These measures will be administered online via RedCap for visits taking place remotely
[d]A modified version of this assessment will be administered for visits taking place remotely
[e] Does not include week 52; for visits taking place remotely, the study medication will be mailed to the subject prior to the visit.

Subjects can withdraw from the study at any time.

D6. Study Drug

Preparation

H_2 can be dissolved in water at atmospheric pressure up to a saturating concentration of 0.8 mM (1.6 ppm), and tissue protection has been demonstrated in a number of rat models using either infusion of H_2-enhanced IV fluids or ad lib access to H_2-enriched drinking water.

To prepare H_2-enriched drinking water, we will use H_2 tablets obtained from the company 'drink HRW'. Drink HRW manufactures effervescent H_2 magnesium tablets for the purposes of creating hydrogen-enriched drinking water. Drink HRW was selected because their tablets reliably produce the intended concentration of hydrogen in the water and remain at a viable concentration long enough for subjects to consume the water. The rapid dispersion of hydrogen is a formidable challenge in the production of hydrogen-enriched water, thus tablets were selected in which we could be assured subjects are receiving the desired amount of molecular hydrogen. Hydrogen water has been "generally recognized as safe" (GRAS) by the Food and Drug Administration (FDA) (GRN 520 available at https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=520&sort=GRN_No&order=DESC&startrow=1&type=basic&search=520. Drink HRW's effervescent H_2 magnesium tablets have been granted "New Dietary Ingredient" status by the FDA (NDI 1104; https://www.regulations.gov/document?D=FDA-2018-S-0023-0162).

The tablets used in this study are sold commercially under the general category of dietary supplements, for the purpose of enhancing the hydrogen dissolved in drinking water (https://drinkhrw.com/shopping/sc_cart/sc_CatTy.asp?SectionID=1&treeUID=1040&guid=60614F5C-6904-4E9A-959B-1448EB4BFCC6). The ingredient in the tablet producing H_2 is magnesium. Each tablet contains 80 mg magnesium, a safe level well below the recommended daily dietary allowance of 420 mg for men/ 320 mg for women. Dissolving one tablet in 250 mL of water will achieve a saturating H_2 concentration of approximately 1.6 ppm. Twice a day subjects will dissolve a tablet into water and drink the effervescent water.

Figure: Nutrition label of drink HRW H2 magnesium tablets

For a matching placebo, we will use effervescent tablets obtained from the company 'drink HRW'. These effervescent placebo tablets also contain 80mg magnesium per tablet, but do not generate H_2-enriched water. The Stony Brook Pharmacy will be responsible for bottling the H_2 tablets and placebo tablets in such a way that study staff and subjects remain blinded. The route of administration and formulation for each dose per patient will be recorded. The study drug will be labeled with the patient's name, Subject identification number, expiration, and the term "Parkinson's study drug - SBUH".

Administration

At the time of the baseline visit, subjects will be provided with H_2 tablets (or placebo tablets) to take home with them. Subjects will be instructed to twice a day dissolve one tablet in 250 mL (8 ounces) of drinking water and then drink the entire glass of effervescent water. Subjects who cannot tolerate thin liquids can use commercial pre-thickened water or commercial thickener to achieve the tolerated consistency (ie. nectar-thick or honey-thick).

Subjects will be instructed to adhere to a roughly q6h – q10h schedule, but for convenience a dose may be taken a few hours earlier or later, as there is no expected mechanism of action indicating that dose timing would be critical for efficacy.

Subjects who are taking routine dietary magnesium supplementation will be advised to suspend this supplementation for the duration of the study, given the presence of magnesium in the H_2 tablets and placebo.

Justification of Dosage

Fu and colleagues found that *ad libitum* administration of 50% saturated H_2-enriched drinking water reduced nigral dopaminergic cell loss in a rat PD model[11]. Furthermore, a recent small pilot trial involving a total of 17 patients with idiopathic PD demonstrated that drinking 1000

mL/day of H_2-enriched water was well-tolerated in PD patients, with possible positive effect on motor symptoms[14].

For these reasons, we have selected a dosage target of 250 mL BID of H_2-enriched drinking water. Duration of therapy is set at 52 weeks.

D7a. Primary Outcome Measures

1. **Safety:** To investigate the safety of H_2-enriched water in PD patients. Safety is defined as the absence of serious adverse events (SAEs) warranting termination of the treatment arm or the trial, as determined by the Data and Safety Monitor.

2. **Tolerability:** To investigate the tolerability of H_2-enriched water in PD patients. Tolerability is defined as the extent to which the treatment group can continue without prolonged treatment suspension (>24 consecutive days or >36 cumulative days) due to adverse events (AEs), as assessed 24 and 52 weeks after receiving the study drug.

The criteria for monitoring safety and tolerability have been modeled after previous phase II clinical trials of antioxidant use in early stage PD patients[9].

D7b. Secondary Outcome Measures

1. **Motor Symptoms**: To investigate the effect of H_2-enriched water on the progression of motor symptoms in PD patients. Progression of motor symptoms will be assessed by change in the motor subscale of the MDS-Unified Parkinson's Disease Rating Scale (MDS-UPDRS-III) from baseline to week 56. This will be assessed in the "off" state.

2. **Quality of life:** To investigate the effect of H_2-enriched water on health-related quality of life in PD patients. Health-related quality of life will be assessed by change in the PDQ-39 from baseline to week 56.

3. **Cognitive Function:** To investigate the effect of H_2-enriched water on the progression of cognitive decline in PD patients. Cognitive function will be assessed using the Montreal Cognitive Assessment (MoCA) scale at baseline and week 56. This will be assessed in the "off" state.

4. **PD Symptom Burden:** To investigate the effect of H_2-enriched water on the progression of symptom burden in PD patients. Progression of symptom burden will be assessed by change in the Total MDS-UPDRS from baseline to week 56.

D8. Enrollment Failure

Subject enrollment will be examined continuously. Our goal is to enroll the targeted 70 subjects within 3 years of enrolling the first subject. If enrollment rate by the end of 18 months is not on

track towards approaching this goal, and no prospects for enhancing enrollment are on the horizon (for example, through partnership with another medical center or network), we will terminate the study and analyze outcomes based upon subjects enrolled at that point. If enrollment is proceeding well, but a reasonable extension of time is needed to complete enrollment of the targeted 70 subjects, we will submit an appropriate amendment request to the IRB prior to the end of the 3 year time frame.

E. STATISTICS

The primary objective of this study will be to assess the safety and tolerability of H_2-enriched water in patients with PD. Safety will be assessed by comparing time to first SAE by log-rank tests. As noted above, safety is defined as the absence of serious adverse events (SAEs) warranting termination of the treatment arm or the trial, as determined by the Data and Safety Monitor. Tolerability will be defined as the extent to which the treatment group can continue without prolonged treatment suspension (>24 consecutive days or >36 cumulative days) due to adverse events (AEs), as assessed 24 and 52 weeks after receiving the study drug. The study is reasonably powered to detect the emergence of an SAE; of note, there were no adverse events or drop-outs in the active treatment arm of a prior pilot study including 9 patients on active treatment with H_2-enriched water[13].

We also plan to perform an exploratory analysis looking for evidence of benefit against disease progression in PD using the secondary outcome measures described above (see D7b). In order to obtain meaningful results towards this end, we have therefore powered the study to detect a superiority of treatment with H_2-enriched water compared to placebo in slowing PD disease progression. A prior pilot study demonstrated changes in the motor subscale of the UPDRS (UPDRS-III) that trended towards significance when comparing active treatment with H_2-enriched water to placebo (-5.7 ± 8.4 vs. 4.1 ± 9.2 for active treatment vs. placebo, respectively). Power calculation using this data suggests that a sample size of 60 will be sufficient to detect a between-group difference with a 2-sided $\alpha = 0.01$ and power = 90%. We plan on a sample size of 70 to account for drop-outs who voluntarily discontinue study treatment and study visits, and are therefore lost to follow-up.

F. FUNDING STATUS, DETAILS

This is an investigator-initiated study that will be funded at launch from the investigators' own uncommitted research funds, possibly with assistance from the Department of Neurology. The investigators may seek outside funding if interim results are encouraging.

G. HUMAN SUBJECTS RESEARCH PROTECTION FROM RISK

G 1. Risk to Subjects

Based on prior studies, we do not anticipate clinically significant adverse effects associated with the administration of H_2-enriched water. Several published studies have administered H_2-enriched drinking water or IV saline to human subjects without observing any serious treatment-associated adverse events. Nakao and colleagues administered 1.5 – 2 L of H_2-enriched water daily for a total of 8 weeks to 20 subjects with potential metabolic syndrome. They observed a small decrease in high density lipoprotein, but no abnormalities in weight, blood pressure, hematological parameters or routine clinical chemistry values[26]. No serious adverse events were noted. Four subjects exhibited mild AEs (1 headache, 1 heartburn, 3 loose stools), but no definitive relationship to treatment could be established. Ishibashi and colleagues administered H_2-enriched IV saline (500 mL qday x 5 days, approximately 1.6 ppm H_2) to 20 normal volunteers to assess safety; researchers reported lack of clinical AEs, and lack of abnormalities detected on routine hematology and blood chemistry[27].

The proposed administration of 250 mL BID of PO water to subjects with Parkinson's disease should not pose significant risk. This volume is less than needed to keep a patient hydrated. Any subject whose attending physician is concerned about study medication-associated fluid overload would be excluded from the study, as per the exclusion criteria.

The risks associated with the blood draw included in this study include temporary pain and bruising there the needle enters the skin, possible fainting, and small chance of infection.

We will ask subjects on parkinsonian medication to refrain from taking their parkinsonian medication for 12 hours prior to the baseline and 56-week visits. This is not expected to contribute any significant risk, as hundreds of studies have been conducted using this procedure. Subjects will sign on the consent form that they understand this procedure and are comfortable with it.

G 2. Adequacy of Protection against Risks

Although the risks of the H_2 therapy proposed here are more than minimal, they should not pose significant risk. The PI and Data Safety Monitor will monitor safety and risks throughout the study (see section G 1).

Signed informed consent will be obtained from each subject. A copy of the consent form countersigned by study staff will be provided to the subject.

The PI and all key personnel involved in the study will have completed the Collaborative IRB training initiative and HIPAA training. The PI will be responsible for reporting adverse events experienced by study subjects to the IRB according to IRB guidelines.

Confidentiality will be maintained. All subject research data will be coded with subject ID number. Paper files will be kept in a secure, locked area. The electronic database used during the study will be secured with a password and saved on a secured shared drive only accessible to key

personnel. Subject results will never be discussed in any form in the presence of other subjects in the study, or with non-laboratory personnel. Subjects' names will not be used in manuscripts or presentations about this research.

G3. Potential Benefits of Proposed Research to the Subject and Others

The proposed therapy may slow the progression of Parkinson's disease, a common neurodegenerative disorder for which disease-modifying treatment is not currently available. Even if the study is negative, observations made during the study may aid the development of future neuroprotective trials.

H. DATA SAFETY MONITORING PLAN

H 1. Adverse events monitoring

Dr. Patricia Coyle, who is unaffiliated with the current study, has volunteered to serve as our data safety monitor (DSM). Dr. Coyle is Professor and Vice Chair (Clinical Affairs) in the Department of Neurology here at Stony Brook. Dr. Coyle will be unblinded to treatment. Assisted by study staff, she will periodically review the protocol documentation and safety data. The first review will occur after the first 5 subjects have been enrolled and on study drug for >6 weeks. Additional reviews will then take place quarterly. Among the important goals of the reviews will be to ensure that adverse events are reported, protocol amendments are filed with the IRB, inclusion/exclusion criteria are followed. If in the DSM's judgement a pattern of unexpected and clinically serious adverse events emerges, or if protocol compliance is inadequate, the study will be terminated.

Any unfavorable and unintended symptom, sign (including a clinically significant abnormal laboratory finding) or disease that is temporally associated with a study will be considered a study AE. The study staff will carefully monitor each subject throughout the study for possible AEs, and document such in an AE log. Minimum information recorded for each AE includes type of event, duration (start and end dates), severity, seriousness, causality to study drug, action taken, and outcome. The PI or one of the Co-Is will grade the severity of AEs based on the DHHS/NIH Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Baseline parkinsonian symptoms and signs documented prior to initiation of study medication will not be considered AEs. The PI will proactively bring to DSM attention any serious AEs judged possibly associated with study medication. Any unexpected fatal or life-threatening suspected adverse reactions will be reported to the FDA within 7 calendar days.

Table 6: Schedule for safety monitoring and assessments

	Assessments	Frequency

H_2 tablet and placebo	Stony Brook pharmacy will bottle tablets following USP 797 guidelines	At each preparation
Study safety	The Data Safety Monitor will review adverse events and protocol compliance	At the first 5 patients enrolled and on study drug > 6 weeks; proactive reviews will then take place quarterly

H 2. Data safety

All paper study data will be kept in secure, locked cabinets. All identifiable electronic study data will be password-protected and saved in Redcap and/or in a secure shared drive on the Stony Brook server only accessible by study staff. The study coordinator will check the data for accuracy. Missing data will be identified and obtained (if possible) in a timely fashion. Study staff will take steps to ensure that all information obtained is kept private. The subjects' names will not be used whenever possible. Study staff will instead use study codes lacking PHI. The electronic data will be banked on the same secure server after the study ends. The study staff may submit a new proposal to obtain approval from the IRB to use this data.

REFERENCES

1. Dorsey ER, Bloem BR. The Parkinson Pandemic-A Call to Action. JAMA Neurol 2018;75:9-10.
2. Dexter DT, Sian J, Rose S, et al. Indices of oxidative stress and mitochondrial function in individuals with incidental Lewy body disease. Ann Neurol 1994;35:38-44.
3. Sian J, Dexter DT, Lees AJ, et al. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 1994;36:348-355.
4. Parkinson Study Group QEI, Beal MF, Oakes D, et al. A randomized clinical trial of high-dosage coenzyme Q10 in early Parkinson disease: no evidence of benefit. JAMA Neurol 2014;71:543-552.
5. Writing Group for the NETiPDI, Kieburtz K, Tilley BC, et al. Effect of creatine monohydrate on clinical progression in patients with Parkinson disease: a randomized clinical trial. JAMA 2015;313:584-593.
6. de Lau LM, Koudstaal PJ, Hofman A, Breteler MM. Serum uric acid levels and the risk of Parkinson disease. Ann Neurol 2005;58:797-800.
7. Ascherio A, LeWitt PA, Xu K, et al. Urate as a predictor of the rate of clinical decline in Parkinson disease. Arch Neurol 2009;66:1460-1468.
8. Schwarzschild MA, Schwid SR, Marek K, et al. Serum urate as a predictor of clinical and radiographic progression in Parkinson disease. Arch Neurol 2008;65:716-723.
9. Parkinson Study Group S-PDI, Schwarzschild MA, Ascherio A, et al. Inosine to increase serum and cerebrospinal fluid urate in Parkinson disease: a randomized clinical trial. JAMA Neurol 2014;71:141-150.
10. Ohsawa I, Ishikawa M, Takahashi K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med 2007;13:688-694.
11. Fu Y, Ito M, Fujita Y, et al. Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease. Neurosci Lett 2009;453:81-85.

12. Fujita K, Seike T, Yutsudo N, et al. Hydrogen in drinking water reduces dopaminergic neuronal loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. PLoS One 2009;4:e7247.
13. Yoritaka A, Takanashi M, Hirayama M, Nakahara T, Ohta S, Hattori N. Pilot study of H(2) therapy in Parkinson's disease: a randomized double-blind placebo-controlled trial. Mov Disord 2013;28:836-839.
14. Yoritaka A, Abe T, Ohtsuka C, et al. A randomized double-blind multi-center trial of hydrogen water for Parkinson's disease: protocol and baseline characteristics. BMC Neurol 2016;16:66.
15. Ohta S. Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. Pharmacol Ther 2014;144:1-11.
16. Fukuda KI, Asoh S, Ishikawa M, Yamamoto Y, Ohsawa I, Ohta S. Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress. Biochem Bioph Res Co 2007;361:670-674.
17. Hayashida K, Sano M, Ohsawa I, et al. Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury. Biochem Bioph Res Co 2008;373:30-35.
18. Terasaki Y, Ohsawa I, Terasaki M, et al. Hydrogen therapy attenuates irradiation-induced lung damage by reducing oxidative stress. Am J Physiol-Lung C 2011;301:L415-L426.
19. Nagata K, Nakashima-Kamimura N, Mikami T, Ohsawa I, Ohta S. Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus-dependent learning tasks during chronic physical restraint in mice. Neuropsychopharmacology 2009;34:501-508.
20. Ostojic SM. Molecular hydrogen: An inert gas turns clinically effective. Ann Med 2015;47:301-304.
21. Nishimaki K, Asada T, Ohsawa I, et al. Effects of Molecular Hydrogen Assessed by an Animal Model and a Randomized Clinical Study on Mild Cognitive Impairment. Curr Alzheimer Res 2018;15:482-492.
22. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181-184.
23. Marks WJ, Jr., Bartus RT, Siffert J, et al. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. Lancet Neurol 2010;9:1164-1172.
24. Abdolahi A, Scoglio N, Killoran A, Dorsey ER, Biglan KM. Potential reliability and validity of a modified version of the Unified Parkinson's Disease Rating Scale that could be administered remotely. *Parkinsonism Relat Disord* 2013;19:218-221.
25. Abdolahi A, Bull MT, Darwin KC, Venkataraman V, Grana M, Dorsey ER, Biglan KM. A feasibility study of conducting the Montreal Cognitive Assessment remotely in individuals with movement disorders. *Health Informatics Journal* 2016, Vol. 22(2) 304–311.
26. Nakao A, Toyoda Y, Sharma P, Evans M, Guthrie N. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome-an open label pilot study. J Clin Biochem Nutr 2010;46:140-149.
27. Ishibashi T, Sato B, Shibata S, et al. Therapeutic efficacy of infused molecular hydrogen in saline on rheumatoid arthritis: a randomized, double-blind, placebo-controlled pilot study. Int Immunopharmacol 2014;21:468-473.