PARTNERS HUMAN RESEARCH COMMITTEE
DETAILED PROTOCOL

Title: Activating the hypoglossal motor nucleus using existing drugs for the treatment of obstructive sleep apnea

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Version Date: December 6th, 2018

I. BACKGROUND AND SIGNIFICANCE

Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder associated with serious cardiovascular and metabolic diseases and with important consequences on the quality of life. It is characterized by repetitive collapse or 'obstruction' of the pharyngeal airway during sleep resulting in a complete closure (apnea) or narrowing (hypopnea) of the upper airway. The severity of the disease is measured as the frequency of obstructive events for each hour of sleep (apnea-hypopnea index, AHI). When OSA is defined as an AHI ≥5 events per hour plus symptoms (eg, daytime sleepiness) or AHI ≥15 events per hour with or without symptoms, the estimated prevalence is approximately 15 percent in males and 5 percent in females[1].

Over the last decade research has shown that a number of pathogenic factors, or traits, contribute to the development of OSA[2-5]. These include: 1) an anatomically small, collapsible upper airway; 2) an oversensitive respiratory control system leading to ventilatory overshoots and undershoots, i.e., instability; 3) a loss of pharyngeal muscle tone or responsiveness during sleep; and 4) a low respiratory arousal threshold, i.e., premature arousal to respiratory stimuli.

Despite our improved understanding of the pathogenesis of OSA, it has not led to improved therapy. Continuous positive airway pressure (CPAP) is still the only viable treatment for most patients, and it is usually effective because it mechanically splints the upper airway open. The problem, however, is that many patients cannot use CPAP because they find it intolerable. This represents a significant health concern, as OSA is known to cause a number of adverse cardiovascular[6-13], neurocognitive[14], and daytime functioning[15] consequences, making OSA a major health concern.

In the presence of an anatomical predisposition to airway collapse, the sleep-related withdrawal of motor output from the hypoglossal motornucleus (HMN) to the upper airway dilator muscles can cause the collapse of pharyngeal structures, with ensuing episodes of reduced ventilation, intermittent hypoxia, hypercapnia, and arousals from sleep[16]. Loss of pharyngeal dilator muscle activation during sleep is central to the pathogenesis of OSA[17]. However, attempts to pharmacologically reverse pharyngeal hypotonia that occurs in sleeping humans have thus far been ineffective mostly because of the lack of agents that can activate receptors *specific to the HMN motor and pre-motor areas*.

Our group has made significant headway in dealing with ventilatory control sensitivity.[18, 19] While a host of oral devices and surgeries have been developed to address the anatomical predisposition to collapse, drugs that activate the pharyngeal muscles are needed.

Recent investigation based on gene expression in the HMN motor and pre-motor (pre-HMN) areas has shown that there is a significant unexplored potential in terms of testing FDA-approved

drugs that can *specifically* target receptors that have *preferential expression on the HMN and pre-HMN areas*. These areas are part of a neural circuit that is critical for OSA pathogenesis because they modulate the upper airway muscle activity during wakefulness and sleep[20]. There is now sufficient data to support a new approach to the development of a pharmacologic treatment for OSA, based on genome-wide analyses of the differential expression of druggable targets in the circuitry controlling motor output to the upper airway musculature.

Our group recently discovered that the combination of atomoxetine (a norepinephrine reuptake inhibitor) and oxybutynin (an anticholinergic) reduced OSA severity by 63% in a group of 20 unselected patients. The putative mechanism of action was an increase in genioglossus muscle responsiveness during sleep by ~3 fold. Based on animal research conducted in Richard Horner's lab in Toronto, norepinephrine is the main neurochemical responsible for upper airway muscles hypotonia during NREM sleep while acetylcholine, through the muscarinic receptors mediate the REM-related atonia in the same muscles. The analysis of the effect of the drugs taken alone revealed that atomoxetine was able to increase the ventilation during sleep compared to placebo but the patients had frequent respiratory and spontaneous arousals from sleep, the sleep quality was compromised and the patients experienced still a high number of hypopneas. Oxybutynin taken alone had only a minor effect in improving ventilation during sleep, however when administered with atomoxetine, it had the effect of consolidating sleep by increasing sleep efficiency, sleep duration and sleep architecture. Based on these findings, we have reasons to believe that oxybutynin could be substituted (in the combination with atomoxetine) with a non-myorelaxing hypnotic free from anticholinergic side effects such as zolpidem, trazodone or gabapentin.

II. SPECIFIC AIMS

AIM 1. To determine the effect of several FDA-approved drugs administered alone or in combination on OSA severity. The drugs tested in this protocol have been selected because of their potential to increase the upper airway muscle activity during sleep by specifically stimulating the HMN and/or the pre-HMN areas in the brainstem.

Specifically, we will test this hypothesis by assessing:

1. The effect of lorcaserin on OSA severity (LTM1201L). Lorcaserin is a weight-loss drug acting specifically as an agonist of 5-HT 2C, a receptor expressed in the pre-HMN area twice as much as the rest of the brain. Previous data collected in an animal model showed that the stimulation of the receptor 5-HT 2C with the agonist 2,5-dimethoxy-4-iodoaminophentamine increased genioglossus activity during sleep 8-fold compared to placebo, and it also reduced the upper airway collapsibility[21].

2. The effect of the combination of lorcaserin and cholecalciferol on OSA severity (LTM1201LD). Cholecalciferol is an agonist of the vitamin D receptor, which is expressed on HMN neurons > 4 times than on other neurons in the brain. Therefore, the vitamin D receptor is highly specific to the HMN. There are no studies assessing the effect of vitamin D administration on OSA severity but several protocols have reported an association between the presence of OSA and vitamin D deficiency[22].

3. The effect of the combination of lorcaserin and nabilone on OSA severity (LTM1201LN). Nabilone is a cannabinoid 1 and cannabinoid 2 (CB1/CB2) receptor agonist approved for the treatment of refractory nausea and vomiting associated with cancer

chemotherapy. Cannabinoid 2 receptor is expressed 6.8 times more on the HMN compared to the rest of the brain. In an animal model, methanandamide – a CB2 agonist – increased tonic and phasic genioglossus activity[23] during wakefulness. Furthermore, a recent clinical trial suggested cannabinoids might be helpful in the treatment of sleep apnea, although the administration of CB1/2 receptor agonist alone had only a modest effect on OSA severity[24].

4. The effect of the combination of lorcaserin and buspirone on OSA severity (LTM1201LB). Buspirone is used in the treatment of generalized anxiety disorder, and it increases the firing in the locus ceruleus. The locus ceruleus is an area of brain where norepinephrine cell bodies are found in high concentration, and it reduces the firing of serotonin-containing neurons. The net result of buspirone actions is that serotonergic activity is suppressed while noradrenergic cell firing is enhanced. The increase in norepinephrine in the brain is important for the HMN area, since it acts as an agonist for the alpha1-adrenergic receptor, which is expressed 3.7 times more on the HMN area compared to the rest of the brain. A previous pilot study suggested a possible modest effect of buspirone administered alone in the treatment of sleep apnea[25].

III. SUBJECT SELECTION

In this pilot study we will recruit a group of 10 OSA patients. These individuals will be otherwise healthy (except for well-controlled hypertension, diabetes, or hyperlipidemia) with no active medical problems and on no medication that could affect respiration or muscle control. Everyone will be 18-70 years of age. Both men and women will have an apnea-hypopnea index (AHI) >10 events/hr during supine NREM sleep. These individuals will be recruited to encompass a large range of AHI's (from 10 to >60/hour).

Exclusion criteria:

- Any medical condition other than well controlled hypertension and mild diabetes. Patients with diabetes will be enrolled only if they are treated with one oral hypoglycemic drug.
- Any medication known to influence breathing, sleep/arousal, or muscle physiology.
- Claustrophobia.
- Inability to sleep supine.
- Allergy to lorcaserin, vitamin D analogues, nabilone, or buspirone.
- History of kidney stones, hypercalcemia, primary hyperparathyroidism, sarcoidosis, hypervitaminosis D, which can be exacerbated by cholecalciferol.
- Individuals with underlying cardiac disease, such as arrhythmias.
- Individuals taking psychiatric medications, such as an MAO-I, SSRI or SNRI, or any of the studied medications for medical care.
- For women: Pregnancy.
- Pulmonary hypertension
- Severe OSA with a mean SaO2 lower than 88%

Equal number of males and females will be recruited. We will consider all applicants regardless of sex, race, color, creed, or national origin.

IV. SUBJECT ENROLLMENT

Men and women with OSA will be recruited in the sleep disordered clinic of BWH and BFH through advertisement flyers. The patients who showed interest in the research will contact us after talking to their physician in the clinic.

Study staff may contact the patient in writing initially and allow the patient to make the first contact if they are interested. Study staff may ask a physician colleague or research nurse to initially explain the study or to re-contact the potential subject after the Investigator has presented the study. Study staff may offer patients the opportunity to take home the Consent Form and call back if they wish to participate. Study staff will recommend that the patient discuss participation with other health care providers.

Subjects from our existing database of OSA patients who previously took part to similar research studies and showed interest in performing more tests will also be contacted by our study coordinator.

Subjects also may be recruited through social media such as Facebook, and bulletin advertisements. Should the subject be interested in the study, they can call the study physician or coordinator to inquire about study participation.

On the phone, subjects will be given a thorough review of the risks, discomforts, potential benefits (if any) and their expected involvement using a prepared script approved by our Institutional Review Board. Subjects will be given a copy of the informed consent and allowed a minimum of 24 hours to review the information and decide on study participation. During this time, the subject will have the opportunity to discuss the research with his/her primary care physician or clinician. The study investigators will be available to answer any questions should any arise. Informed consent will be obtained from all subjects by a licensed physician investigator prior to commencement of any study procedures.

Inclusion and exclusion criteria will be carefully assessed prior to enrollment. Assuming subjects meet the inclusion criteria, they will begin the protocol by scheduling their overnight studies in the clinical/physiology laboratory. Subjects will be informed that they may withdraw from the study at any point, with no impact on their ongoing care.

All email sent outside of the Partners firewall containing confidential information will be encrypted using "Send Secure" as per the Partners Policy. Subjects may opt out of encrypted email communications if they have been advised of the risks associated with unencrypted email, and they indicate a preference to receive unencrypted email despite the risks. Consent to receive unencrypted email will be documented per the Partners Policy in the subject's record. We will discourage subjects from communicating about medical issues by non-secure email.

V. STUDY PROCEDURES

Protocol

After a medication and screening visit, five overnight sleep studies will be performed approximately 1 week apart in an open-label randomized design. This study has been designed to quickly generate preliminary data for future studies, targeted to the most effective

combinations of drugs that will be performed in a double-blinded fashion in a higher number of patients.

Consent and medication visit

The study will be explained in detail and an assessment will be made by an investigator to ensure the study procedures can be performed and written consent will be obtained. If the subject enrolled is a premenopausal woman, we will perform a urine pregnancy test at this visit.

Sleep Studies

For each night, the subjects will arrive at the sleep laboratory at approximately 6:30pm. A physician will perform a history and physical examination. The placebo or drug(s) will be administered 30 minutes before lights out. The patients will perform five sleep studies in random order:

1) placebo night
2) lorcaserin (15mg) night – LTM1201L
3) lorcaserin (15mg) +cholecalciferol (1500 IU) night – LTM1201LD
4) lorcaserin+nabilone (1mg) night – LTM1201LN
5) lorcaserin (15mg) + buspirone (20mg) night – LTM1201LB

Measurements and equipment:

Subjects will be instrumented with standard polysomnography (PSG) recording sensors. Sleep stage and arousals will be measured with electrodes pasted on to the scalp, face, chin, and chest (EEG, EOG, EKG, chin EMG). Paste-on EMG electrodes will be placed over the anterior tibialis muscle to detect leg movements. Respiratory effort belts will be placed around the chest and abdomen to measure breathing movements. Oxygen saturation will be measured continuously with a pulse oximetry probe placed on the fingertip. Snoring will be detected with a small microphone positioned over the suprasternal notch. Body position will be recorded with a sensor taped to the thoracic belt. Each of these devices is standard for diagnostic PSG and should not be uncomfortable.

To measure airflow, a standard CPAP mask will be placed over the nose and held in place with straps. The mask allows monitoring of breathing (inspiratory flow by pneumotachograph that can be integrated to yeld tidal volume) and expired carbon dioxide levels (PCO_2) using a calibrated infrared CO_2 analyzer (Capnograph/Oximeter Monitor).

After all the equipment is in place, the patient will be asked to sleep in the supine position for at least one half of the night (4 hours). As much data will be recorded from NREM and REM sleep as possible over the night. Following completion of the study, all equipment will be removed, and the subject will be able to sleep in the laboratory free from equipment for the rest of the night. Alternatively, if the subject feels alert enough to leave, they may do so.

Data Analysis:

Apneas, hypopneas, arousals, and sleep stages will be scored using standard American Academy of Sleep Medicine guidelines[26] by a registered polysomnographic technologist (RPSGT) blinded

to the treatment allocation. Hypopneas will be defined as reduction in flow ≥30% from baseline, lasting at least 10 seconds and associated with an arousal from sleep or an oxyhemoglobin desaturation ≥ 3%.

If the data collected are considered insufficient, the subject may be asked to repeat the whole study or a part of it.

VI. BIOSTATISTICAL ANALYSIS

In this protocol, we want to collect pilot data to measure the possible effect size of this intervention. Variables of interest will be compared using a mixed effect model with $p<0.05$ considered as statistically significant.

VII. RISKS AND DISCOMFORTS

We believe that the risks associated with participation in this study are small since the drugs are already FDA-approved for other purposes and we are only performing standard clinical sleep studies. All study procedures have been conducted in our laboratory without serious incident. Anticipated risks and discomforts are listed below:

1. The equipment used for assessing sleep (paste on electrodes) is standard and poses no risk. The electrodes may be mildly uncomfortable and could cause some sleep interruption. Thus, subjects may feel somewhat tired the day following this study.

2. Lorcaserin side effects include: headache (15% to 17%), hypoglycemia (diabetic patients 29%; symptomatic 7%; severe: 2%), back pain (6% to 12%), upper respiratory tract infection (14%), nasopharyngitis (11% to 13%), hypertension (5%), peripheral edema (5%), decreased heart rate (less than 50 bpm: 4% to 5%), acquired valvular heart disease (3%), dizziness (7% to 9%), fatigue (7%), anxiety (4%), insomnia (4%), depression (3%), stress (3%), cognitive dysfunction (2%), psychiatric disturbance (2%), exacerbation of diabetes mellitus (3%), nausea (8% to 9%), diarrhea (7%), constipation (6%), xerostomia (5%), vomiting (4%), gastroenteritis (3%), toothache (3%), decreased appetite (2%), urinary tract infection (7% to 9%), Decreased hemoglobin (10%), decreased neutrophils (6%), muscle spasm (5%), musculoskeletal pain (2%), cough (4% to 8%), oropharyngeal pain (4%), sinus congestion (3%)

3. Cholecalciferol: no adverse reactions are listed in the manufacturer's labeling. High levels of Vitamin D may facilitate kidney stones formation and atherosclerosis, secondary to hypercalcemia. There are no known interactions between cholecalciferol and lorcaserin.

4. Nabilone (Cesamet) side effects include: drowsiness (52% to 66%), dizziness (59%), vertigo (52% to 59%), euphoria (11% to 38%), ataxia (13% to 14%), depression (14%), lack of concentration (12%), sleep disorder (11%), xerostomia (22% to 36%), visual disturbance (13%), hypotension (8%), dysphoria (9%), headache (6% to 7%), sedation (3%), depersonalization (2%), disorientation (2%). There are no known interactions between nabilone and lorcaserin.

5. Buspirone side effects include: Dizziness (3% to 12%), Chest pain (≥1%), Drowsiness (10%), headache (6%), nervousness (5%), confusion (2%), excitement (2%), numbness (2%), outbursts of anger (2%), abnormal dreams (≥1%), ataxia (1%) paresthesia (1%), Diaphoresis

(1%), skin rash (1%), Nausea (8%), diarrhea (2%), sore throat (≥1%) Weakness (2%), musculoskeletal pain (1%), tremor (1%), Blurred vision (2%), Tinnitus (≥1%), Nasal congestion (≥1%). There are no known interactions between buspirone and lorcaserin. Both these agents are serotonin modulators but the development of serotonin toxicity/serotonin syndrome is highly unlikely because buspirone inhibits the release of serotonin in the brain while lorcaserin specifically stimulates a single serotonin receptor (5HT 2C).

VIII. POTENTIAL BENEFITS

Although it is unlikely that there will be any direct physical benefit to the subjects from participating in this study, we will make known to each subject, if requested, some of the information we have gathered from this testing. This study provides a unique opportunity to gain insight into the specific mechanisms by which these novel drugs may improve upper airway muscle function. The results may, in the future, lead to improved strategies for the treatment of sleep apnea. However, if previously unknown abnormalities of sleep and breathing are encountered, this information will be passed onto the subject. Results can be forwarded to the primary care physician or clinician at the request of the subject.

IX. MONITORING AND QUALITY ASSURANCE

As these studies represent physiologic investigation and not clinical studies, no formal quality assurance programs will be implemented. However, the ongoing results, problems, and limitations of the study will be presented on a regular basis to the investigators in the Division of Sleep Medicine. Also, any adverse events will be promptly reported to the Human Research Committee for review. See attached Data and Safety Monitoring Plan.

We will also elect an independent safety monitor (ISM) to review the study and protocol on a quarterly basis. The designated safety monitor is Robert Thomas, MD. Dr. Thomas is an Associate Professor in Medicine at Harvard Medical School and practices out of Beth Israel Deaconess Hospital in Boston. He specializes in Sleep Medicine (Circadian Rhythm Disorders, Complex Sleep Apnea, Insomnia, Restless Leg Syndrome, Periodic Limb Movements, and Narcolepsy). He is not directly associated with our research lab. He has no say over the design or implementation of these studies and will review the practices in an unbiased manner.

X. REFERENCES

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