DETAILED PROTOCOL

Version: October 17, 2016

Title: Pharmacologically improving the pharyngeal muscle activity during sleep: implications for the novel treatment of obstructive sleep apnea.
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I. BACKGROUND AND SIGNIFICANCE

Obstructive sleep apnea (OSA) is characterized by repetitive collapse or 'obstruction' of the pharyngeal airway during sleep. Over the last decade, and particularly in the last few years, research has shown that a number of pathogenic factors, or traits, contribute to the development of OSA (1-4). 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 the 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 (5-12), neurocognitive (13), and daytime functioning (14) consequences.

One possible approach to finding alternative treatments for OSA is to continue searching for a single drug or agent that, like CPAP, has a large enough effect size to override the various causes of OSA. This approach has proven difficult, and while we hold out hope for such a drug, in the meantime we have adopted another (possibly equally effective) tactic, which is to *target the relevant upstream mechanisms or individual traits with one or more drugs in an individual patient*. The rationale for this approach is that non-CPAP therapies have tended to have small effect sizes and proven, by themselves, marginally effective at fully correcting OSA in many patients. Another reason stems from the manner in which most major medical disorders are treated. Rarely is a single drug used to treat, for example, congestive heart failure, hypertension, asthma, etc., unless the condition is mild. In our view, a similar approach should be taken for managing OSA.

Regardless of whether single or multidrug therapy is ultimately used, *the search for alternative treatments has been lacking a very important ingredient – a drug to stimulate the pharyngeal muscles*. While a host of oral devices and surgeries have been developed to address the anatomical predisposition to collapse, and our group has made significant headway in dealing with ventilatory control sensitivity (15, 16), drugs that activate the pharyngeal muscles are needed. Interestingly, new research in animals has improved our understanding of the state-dependent neurotransmitters involved in pharyngeal muscle activation during sleep. Importantly, the loss of noradrenergic activity is now thought to play the key role in the sleep-related hypotonia of pharyngeal muscles.

Chan and colleagues (17) showed in rats that the noradrenergic antagonist terazosin substantially reduced genioglossus (a major muscle of the upper airway) activity (EMG_{GG}) during wakefulness and produced REM-like atonia during NREM sleep, illustrating the importance of noradrenergic mechanisms. Other studies (18, 19) also support the notion that progressive withdrawal of noradrenergic tone, from wakefulness to NREM and REM sleep, is the major mechanism causing sleep-related pharyngeal hypotonia. While noradrenergic withdrawal is thought to be the main cause of pharyngeal hypotonia in NREM sleep, there are additional mechanisms that cause further reduction in REM sleep. Chan and colleagues (17) failed to reverse REM atonia with alpha-1 receptor agonists applied to the hypoglossal nucleus, suggesting that another, possibly inhibitory, mechanism is at work. Horner and colleagues have

identified this inhibitory process as muscarinic by demonstrating restoration of EMG_{GG} activity during REM sleep with the muscarinic antagonist scopolamine (20, 21). More recently, these researchers found that the multiple state-dependent adrenergic, serotonergic and muscarinic systems produce suppression of EMG_{GG} activity during sleep via a convergent ionic mechanism: increased potassium conductance. Blockade of potassium channels has been shown in mice to be capable of reactivating the pharyngeal musculature throughout sleep (22).

However, due to the only recent identification of this process, there has not yet been an attempt to stimulate the pharyngeal muscles with noradrenergic drugs in sleeping humans. Now, more than ever, the stage has been set for stimulating the pharyngeal muscles across both NREM and REM sleep.

II. SPECIFIC AIMS

To determine the effect of noradrenergic and antimuscarinic on genioglossus activity and OSA severity. We hypothesize that existing drugs with these neurotransmitter profiles could (partially) restore pharyngeal muscle activity in humans during sleep.

Specifically, we will test this hypothesis by assessing;

1. The effect of pseudoephedrine (an α1-adrenergic agonist) combined with diphenydramine (an antihistaminic with sedative properties and with antimuscarinic action) on EMG_{GG} during sleep (PART D).

III. SUBJECT SELECTION

We will recruit a group of subjects with OSA. These subjects will be recruited from the community and be between 21-65 years old. The purpose of studying patients with OSA is to determine what effect this drugs will have on improving the patient's OSA severity.

a) Obstructive Sleep Apnea Patients (n= 15): Patients with OSA will be recruited from our clinical sleep laboratory at Brigham and Women's Hospital, as well as from our existing database of OSA patients. These individuals will be otherwise healthy (except for well-controlled hypertension; defined as systolic blood pressure <140 mmHg and diastolic <90 mmHg) with no active medical problems and on no medication that could affect respiration or muscle control. All will be 21-65 years of age. Both men and women will have an apnea-hypopnea index (AHI) >15 events/hr during supine NREM sleep. These individuals will be recruited to encompass a large range of AHI's (from 10 to >60/hour).

b) *Exclusion criteria:*

- Any active medical condition other than well controlled hypertension, hyperlipidemia, gastroesophageal reflux disease, depression.
- Any medication known to influence breathing, sleep/arousal or muscle physiology.
- Claustrophobia.
- Inability to sleep supine.
- Allergy to lidocaine, Oxymetazoline HCl, pseudoephedrine HCl, diphenhydramine.
- Individuals with underlying cardiac disease, such as arrhythmias.
- History of seizures
- History of moderate or severe renal impairment
- For women: Pregnancy.

We will consider all applicants regardless of sex, race, color, creed, or national origin.

IV. SUBJECT ENROLLMENT

Subjects will be recruited through email, telephone, newspaper, and or bulletin advertisements. Men and women with OSA will be recruited from a pool of patients being newly diagnosed with OSA and currently followed in our outpatient clinic or by advertisement in the sleep disorders clinic. Only patients who have stated in the initial clinical questionnaire that they are interested in hearing about research studies will be contacted by phone. Should the subject be interested in the study, they can call the study physician or coordinator to inquire about study participation. We will also recruit from our existing database of research participants.

Subjects who respond will be given a thorough review of the risks, discomforts, potential benefits to the study 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 make a decision 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 by the Principal Investigator or an experienced co-investigator prior to participation in the study. The opportunity to talk to a licensed MD (who is readily available at the time of consent, and available overnight at the hospital) will be offered in each case. Subjects will have more than 24 hours to consider participating in the study. Any consent issues / problems will be reported to the PHRC in real time rather than waiting to report at the time of Continuing Review

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 laboratories. Subjects will be informed that they may withdraw from the study at any point, with no impact on their ongoing care. We have not previously had difficulty enrolling participants into similar studies performed in our laboratory.

If the data collected will be considered insufficient by the PI or by the co-investigators, the subject will be asked to repeat the whole study or a part of it without signing a new informed consent form.

V. STUDY PROCEDURES

Protocol:
Two overnight sleep studies will be performed approximately 1 week apart: a placebo night and a pseudoephedrine-plus-diphenhydramine (120+50mg PO, DAW1033D) night, in double-blinded randomized control design. For each night, the subjects will arrive at the sleep laboratory at approximately 7:00pm. A physician will complete a complete physical and medical history. The placebo or DAW1033D will be administered 30 minutes before lights out. At least 15 minutes of quiet wakefulness will be recorded to quantify the subject's awake EMG_{GG} activity. Subjects will then be allowed to fall asleep during which we will assess EMG_{GG} activity throughout the rest of the evening (see below for details).

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 either the fingertip or earlobe. 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.

One nostril and the back of the throat will be anesthetized with 4% lidocaine; only 2–3 ml (80–120 mg) of lidocaine is used. One small, flexible pressure-tipped catheter (Millar) will then be inserted through the anesthetized nostril until the tip of the catheter is located just above the epiglottis (or in the esophagus) by visual inspection through the mouth; the catheter is then taped to the nose to prevent movement. This catheter is used to assess upper airway negative pressure and to calculate airflow resistance both awake and during sleep.

Electromyogram activity from the genioglossus (EMG_{GG}) muscle (a major upper airway dilator muscle) may also be recorded using unipolar intramuscular electrodes as described in our previous studies (1, 23, 24). Two 25 gauge needles containing 30 gauge, Teflon-coated stainless steel wires are inserted into the muscle after topical anaesthesia with 4% lidocaine. A maximum of 2 mls of lidocaine will be used for needle insertion. If the patient cannot tolerate needle insertion once the maximum dose of lidocaine has been applied, then the procedure will be stopped. The needle is immediately removed leaving the wire in place. Both electrodes are referenced to a single ground producing a bipolar recording. GG needle placement is as follows: The muscle is approached through the floor of the mouth with each needle being inserted about 3-5 mm lateral to the frenulum and about 12-15 mm into the body of the genioglossus near its insertion into the mandible. Once the electrodes are placed, the EMG signal is amplified, rectified, and integrated on a moving time average basis with a time constant of 100 msec (CWE Incorporated).

The subject is then asked to lay down (supine) and a comfortable sealed mask will be placed over the nose and held in place with straps. The mask allows monitoring of breathing (inspiratory flow by pneumotachograph which can be integrated to tidal volume) and expired carbon dioxide levels (PCO_2) using a calibrated infrared CO_2 analyzer (Capnograph/Oximeter Monitor), and if available, end-tidal oxygen levels using a calibrated O_2 analyzer.

Tests performed during wakefulness.
The patients, with the described monitoring equipment in place, will undertake two tests before going to sleep for the night, although some of the testing may not be performed in all subjects depending on availability of equipment, scheduling issues and willingness of the subject to undergo all of the testing.

1. Upper airway muscle activity during wakefulness
The subject will be asked to perform several maneuvers to assess the activity of the GG during wakefulness. The maneuvers include a maximal tongue protrusion, inspiration against a closed airway, swallow and maximum voluntary ventilation. Each of these maneuvers will be repeated 3 times.

After these test during wakefulness, the subjects will be given Desipramine (200 mg PO) /placebo to take and then be connected to a positive/negative pressure source (Philips-Respironics, Murrysville, PA) to enable rapid switching between pressure levels and allowed to fall asleep on 4 cmH_2O of CPAP. When stable sleep is reached, the CPAP will be modified to the level that abolishes flow limitation, as determined by the airflow waveform. Five minutes of stable sleep will then be recorded. Following this baseline recording period, the CPAP level will be reduced to varying suboptimal pressures for several minute

intervals to see how the genioglossus responds to airway collapse. In the case of controls, the suboptimal level required to collapse the airway may require negative pressures. If the subject awakens during the suboptimal pressure drop, the pressure will be immediately returned to the holding pressure until stable NREM sleep is again achieved. We have performed this technique in numerous healthy controls and OSA patients with no complications to date. Furthermore, it is our experience that the response to negative pressure drops which is often used in healthy controls is similar to the suboptimal positive pressures used in OSA patients.

After approximately 4 hours of sleep, we will remove the CPAP and ask the subjects to sleep in the lateral position for the duration of the evening to minimize pharyngeal resistance similar to previous studies of this kind (25). 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:
Muscle activity will be quantified using standard procedures described in our previous studies (1, 23, 24). Briefly, the raw EMG_{GG} signal will be rectified and smoothed with a 100 msec window. The peak phasic, as well as tonic, activity of the smoothed signal will be identified for each breath. Data from quiet wakefulness (free of swallowing and movement artifacts) will be averaged to determine the peak phasic and tonic activities during this state. The same will be done for stable NREM and REM sleep (free of arousals and other artifacts). Both NREM and REM sleep will be analyzed, recognizing that REM is less frequent on these drugs. Pharyngeal muscle responsiveness will be determined by calculating the slope and intercept of the epiglottic pressure versus muscle EMG relationship.

Reimbursement
Subjects will receive $100/night for participation in each overnight study (TOTAL = $200 for each PART of the study). Reimbursement for parking expenses will be provided.
If the subjects will repeat a part or the entire protocol because of insufficient data collection, they will be reimbursed $100 for any extra night.

VI. BIOSTATISTICAL ANALYSIS

Subjects will be prospectively enrolled until 10 had completed both study nights. Sample size was chosen to facilitate detection of a clinically-important 50±50% reduction in AHI. Calculations were based on previous studies conducted in our laboratory. To account for a ~30% failure rate approximately 13 patients will be enrolled in the study.
In order to reduce the influence of potential outlier, data will be expressed as median [interquartile range] and data on placebo vs DAW1033D will be compared using Wilcoxon test. A p value < 0.05 will be considered as statistically significant.
In the pilot study using the noradrenergic drug desipramine, the average difference in EMG_{GG} activity (between the placebo night and the drug night, with EMG_{GG} quantified as a percentage of wakefulness) was 47 ± 20%. In order to detect this difference with 80% power and a 5% level of significance, 6 subjects would need to be studied.

VII. RISKS AND DISCOMFORTS

We believe that the risks associated with participation in this study are minimal. 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. Pharyngeal Pressure Catheter: Inserting the pressure catheter through the nostril and into the pharynx may be uncomfortable and could cause gagging or even vomiting. The anesthetized and fasting state substantially reduces the risk of discomfort or complications. As the subject will have a catheter in his/her pharynx and a nasal mask in place, the risk of aspiration must be considered. However, this has never been encountered after hundreds of similar studies. The catheter, once in place, does not gag the subject and the mask can easily be removed. Overall, the risk of aspiration is minimal.
    i. Lidocaine. If the subjects have any history of lidocaine allergy, they will be excluded from the study. Excessive use of lidocaine can cause seizures, but this is reported with much higher doses than will be utilized in this study. However, subjects will be informed that lidocaine has an unpleasant taste.

3. Intramuscular EMG Determination: Needle insertion may be painful although the mucosa is topically anesthetized with lidocaine. We have found this pain to be similar to venipuncture and well tolerated. Discomfort is minimal once the needle is removed and the wire is left in place. A small amount of bleeding and/or a small bruise may occur. Both the needles and wires are sterilized. However, intramuscular or surface infection could occur, as it is impossible to sterilize the inside of the mouth. We have never encountered this problem or heard of it occurring elsewhere. There may be some residual soreness in the tongue for several hours after the wires are removed.

4. Pseudoephedrine: this drug is available at the dose of 15, 30, 60 and 120 mg as over-the-counter medication of nasal congestion and cold relief. Common side effects include arrhythmia, tachycardia, palpitations, nervousness, excitability, restlessness, dizziness, weakness, insomnia, headache, drowsiness. Other side effects may include anorexia, constipation, diarrhea, hypertension, dry throat, ischemic colitis, nausea, vomiting, xerostomia, dysuria, polyuria, urinary retention, tremor, weakness, blurred vision, diplopia, dry nose, dyspnea, nasal congestion, thickening of bronchial secretions, wheezing, tinnitus.

5. Diphenhydramine: this drug is available at the dose of 25 and 50 mg as over-the-counter medication of allergy and cold relief. Common side effects include: sedation, sleepiness, dizziness, disturbed coordination, epigastric distress, thickening of bronchial secretions. Other side effects may include extrasystoles, hypotension, palpitations, tachycardia, blurred vision, diplopia, difficulty in micturition, urinary frequency, urinary retention, tinnitus, anorexia, constipation, diarrhea, dry mucous membranes, epigastric distress, nausea, vomiting, xerostomia.

6. Combination of pseudoephedrine and diphenhydramine: the association of these drugs is already available (at a lower dose of 60/25 mg) as over-the-counter medication for nighttime relief of allergy, sinus and cold symptoms (see for example Benadryl Allergy Sinus). The most common side effects of the combination of the drugs are constipation, diarrhea, dizziness, drowsiness, dry mouth, nose, and

throat; excitability, headache, anorexia, nausea; nervousness or anxiety, insomnia, weakness. As both drugs are commonly administered at higher doses alone, we believe that increasing the dose of the combination will not determine excessive increase in the risk of serious side effects. Furthermore, the drugs will be administered for only one night in a monitored setting: an experienced researcher will be present for the entire night checking vital signals in real time (heart rate, oxygen SaO2, EEG), the patient will be under audio and video-surveillance. An MD will be available for the duration of the study and the following morning if necessary.

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 physiologic 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

We will follow the Data and Safety Monitoring Plan included as an attachment. As this study is a physiological investigation and not clinical trial, a formal Data and Safety Monitoring Board will not be implemented. The PI will be responsible for monitoring safety and quality assurance. Additionally, 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. Any adverse events will be promptly reported to the Human Research Committee for review according to HRC guidelines.

Adequacy of Protection Against Risks

All of our laboratory personnel involved in the research of human subjects have completed the required institutional program for education in the protection of human research participants and their confidentiality. The institutional educational program consists of the review of regulatory and informational documents pertaining to human-subject research, passing a test demonstrating knowledge of the ethical principles and regulations governing human-subject research and signing a statement of commitment to the protection of human subjects.

All electronic data will be stored on secure computers under password protection with no access allowed to individuals outside of our research team. All paper data will be stored under lock and key with access only given to the study staff.

Protection Against Risks

We believe that all possible safeguards are in place to minimize the risk. However, several steps will be taken to insure patient comfort and safety. We will work with our IRB to come up with a safety monitoring plan to minimize risk and discomfort. This will include:

- Reporting any complications of our studies immediately to the IRB.

- Appoint a safety officer (David Andrew Wellman, MD) who will work with our physicians and technicians to maximize safety and comfort.
- Our study coordinator will call each subject 2-3 weeks after the study to determine if any problems resulted from the study.

The study coordinator will meet with the safety officer and PI monthly (and as needed) to go over any complaints or problems. The safety officer will call the patients with problems directly to verify important issues. If problems are identified, the protocol will be adjusted as needed. Based on conversations with the NIH and the NHLBI policy (http://www.nhlbi.nih.gov/funding/ethics.htm), we will not require a formal data safety monitoring board. However, we do have a thorough data safety monitoring plan whereby our safety officer will review all adverse events in order to classify them as serious adverse events, minor adverse events, and whether they are anticipated or unanticipated, and study related or unrelated as per our IRB rules and the NHLBI policy. The medical monitor will be an academic physician with considerable experience in clinical research but not involved in our research program or a co-investigator in any of our studies (Dr. David White). The medical monitor will strictly adhere to the following definitions:

Definitions

Definitions are per January 2007 OHRP *Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events, OHRP Guidance,* http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm

Adverse Event (AE): any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Serious adverse event (SAE): any adverse event that:

- Results in death
- Is life threatening, or places the subject at immediate risk of death from the event as it occurred
- Requires or prolongs hospitalization
- Causes persistent or significant disability or incapacity
- Results in congenital anomalies or birth defects
- Is another condition which investigators judge to represent significant hazards

Unanticipated Problem (UP): any incident, experience, or outcome that meets all of the following criteria:

- unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;
- related or possibly related to participation in the research, in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research;
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Adverse Events (FDA) versus Unanticipated Problems (OHRP)

- All adverse events are not necessarily unanticipated problems
- All unanticipated problems are not necessarily adverse events

- Some events may be both

X. REFERENCES

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