THE ROLE OF OREXIN IN HUMAN PANIC DISORDER

PROTOCOL

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BACKGROUND AND SIGNIFICANCE

Orexins

Hypocretins (orexins), a more recently identified class of pro-arousal neuropeptides, are synthesized by neurons in the lateral and posterior hypothalamus.[1] The main described orexins, orexin A (ORX A) and orexin B (ORX B), are both cleaved from a common precursor peptide, prepro-orexin.[2] Orexin A, a 33 amino-acid residue peptide, appears to be conserved across many mammalian species. Orexin B contains 28 amino acids. Orexins promote a variety of behaviors including alertness, vigilance, locomotion, fight-flight responses, and feeding. The physiological effects of the orexins are mediated via 2 G-protein coupled receptors, ORX1 and ORX2.[3,4] Orexin A binds with greater affinity to the ORX1 receptor, while orexin A and B bind with similar affinity to the ORX2 receptor.

Orexins and Animal Fear Models

Orexins have been implicated in anxiogenesis in some animal fear models. For example, central (icv) injection of orexin A in mice induced anxiety-like responses in the light-dark exploration test and elevated plus maze.

Using an established, γ-aminobutyric acid (GABA)-deficit, rodent model of panic-vulnerability,[5,6] our Indiana University preclinical anxiety collaborators provoked a panic-like response in rodents with an anxiogenic sodium lactate (NaLac) infusion, which response was blunted following either site-specific orexin (ORX) gene silencing or systemic pretreatment with an ORX1 antagonist.[7]

In addition, ORX neurons (peptidergic neurons in the lateral hypothalamus) were shown, in turn, to stimulate discrete efferent sites within an emotional network (e.g., bed nucleus of the stria terminalis) to elicit specific behavioral components of the panic-response following sodium lactate. Taken together, these results support the concept that ORX hypersecretion or ORX neuronal overactivity could also be present in human PD.

Orexins' Emerging Role in Human Anxiogenesis / Panicogenesis

Similar to the NaLac model animals, humans with PD have been reported to have cortical and subcortical GABA deficits.[8-11] If these GABA deficits also extend to impairment of GABAergic inhibition of DMH ORX neurons in PD patients, as predicted by the NaLac animal model, they may result in ORX hypersecretion, increased sympathetic activation, and panicogenesis.

There have been few clinical studies of ORX metabolism or function in human anxiety populations. However, we have recently generated human pilot data in our laboratory, studying the effects of a well-documented anxiogenic stimulus (35% CO_2 inhalation) on behavioral, physiological, and biochemical (plasma ORX A; assayed by a standard RIA kit) measures, in 1 PD patient and 2 healthy volunteers (see Table 1, below). In this paradigm, the PD patient had a mild panic episode associated

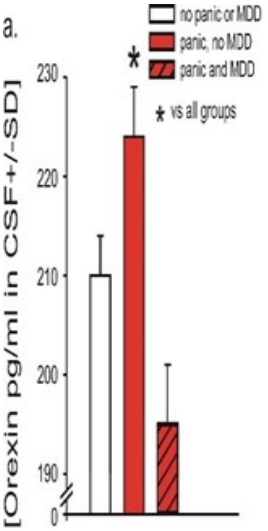
with marked early elevations in plasma ORX levels, relative to the volunteers who had minimal anxiety, consistent with a role for ORX in the initiation or elaboration of the human panic response.

Table 1. Plasma ORX Responses to 35% CO_2 Challenge

Plasma Orexin (ORX A) Levels (in pg/ml)			
Time (mins):	**Baseline**	**+1**	**+15**
PD #1	23.6	73.1	26.2
Vol. #1	34.8	53.2	29.4
Vol. #2	43.3	34.3	40.1

We also demonstrated that, in contrast to human subjects without any axis I psychiatric disorder or with depression alone without panic, only subjects who had high panic scores but no depression had significantly elevated CSF ORX levels[7] (see Figure 1, below).

Figure 1. Panic symptomatology associated with elevated CSF ORX in suicide attempters



b.

Subject group	No panic/No MDD (N=28)	Panic/No MDD (N=12)	Panic and MDD (N=13)
Age in years (mean+SD)	35+11	38+15	45+15
Gender (M:F)	17:11 61% :49%	3:9 25%:75%	7:6 54%: 46%
Co-morbid axis I diagnosis	Gen. Anxiety (3) Adjustment disorder (17) Dysthymia (4) Depressive syndrome NOS (4)	Gen. Anxiety (2) Adjustment disorder (2) Dysthymia (4) Depressive syndrome NOS (4)	MDD (13)

The ORX hyperactivity hypothesis of panic that has been evinced from this work is highly innovative, and promises to broaden our understanding of the neurobiology of human panic disorder (PD), as well as provide new treatment directions.

While there are limitations with using plasma ORX A as a measure of CNS ORX function, one research group has recently published human data indicating that resting state CSF and plasma ORX A levels are highly correlated.[12]

Accordingly, the central hypothesis of this translational human pilot project, and a more definitive project based on it, is as follows: PD is a human anxiety disorder associated with specific cortical and subcortical GABA deficits that result in disruption of normal inhibitory regulation of pro-arousal ORX neurons. This disruption promotes excessive ORX release, sympathetic activation,[13] and vulnerability to spontaneous or chemically induced panic. Pretreatment with an ORX1 receptor antagonist[14,15] prior to chemical challenge, as proposed in this submission, is therefore expected to block the evoked panic response.

Rationale for the Use of CO_2 Inhalation

The 35% CO_2 challenge is well documented in the literature as being reliable, safe, and easy to administer.[16,17] The procedure has acceptable test-retest reliability,[18] and may be used to monitor improvement in clinical status following the administration of antipanic medications.[19]

Approximately 70% of PD patients will have a panic attack in response to this challenge, which closely resembles a real-life panic.[20] Therefore, in addition to resting/baseline measurement of plasma ORX A, we also will examine CO_2-evoked levels of plasma ORX A in PD patients, and we will correlate these responses with other behavioral and physiological parameters recorded during the CO_2 test. The PI has had considerable experience using PD challenge paradigms in clinical research contexts, and he is very familiar with the application of the 35% CO_2 challenge.[21,22]

PROJECT AIMS AND EXPECTED RESULTS

The project is a 1-year study that gathers pilot data relating to the role of orexin in human panic disorder. The effect sizes generated from this pilot work will permit planning and powering of a larger-scale study. It is anticipated that we will complete the study over the course of one year.

Specific Aim 1 will be to provide a preliminary demonstration that acute administration of the first-in-class, FDA-approved insomnia agent, suvorexant,[14,23] a mixed ORX1/2 receptor antagonist, will block 35% CO_2-induced panic symptoms in panic disorder (PD) patients, via amelioration of central ORX neuronal hyperactivity (as reflected in blunted plasma ORX responses to CO_2 challenge).

To address **Specific Aim 1,** we will use a prospective, parallel-group, repeated-measures design to compare behavioral, physiological, and biochemical (plasma ORX) responses in 2 independent, unmedicated groups of PD outpatients (n=6 in each group) at baseline/resting state and after panic provocation due to brief (1 minute) inhalation of a 35% CO_2 / 65% O_2 gas mixture. PD patients will be randomized, in a double-blind manner, to receive either a single, oral dose of the mixed ORX1/2 receptor antagonist, suvorexant (10 mg dose), or identical placebo, 120 minutes before CO_2 challenge.

Exploratory aim: To acquire DNA samples for future candidate gene/DNA analysis. Genes of interest with respect to ORX metabolism are the preproORX gene and genes that code for the receptors ORX1 and ORX2.

Expected results: It is expected that, compared to placebo, suvorexant pretreatment will blunt behavioral, physiological, and biochemical (plasma ORX) responses to 35% CO_2 in PD, due to suppression of CNS ORX hyperactivity. The effect sizes generated from the pilot work will permit planning and powering of a larger-scale study, to definitively address Specific Aim 1.

INNOVATION

The current work promises to determine the human applicability of a novel pathophysiologic concept generated from preclinical science, in this instance the ORX hyperactivity hypothesis of panic/anxiety. Our proposed study will perform rapid clinical testing of the ORX hypothesis with a view to identifying a new class of anxiolytic agents (ORX1 antagonists).[15]

This translational, experimental medicine approach is expected to hasten proof-of-concept testing of novel anxiolytics for PD and for all anxiety syndromes where panic attacks are an important clinical feature. Not only does the ORX hyperactivity hypothesis promise to inform new treatment development, but it may also shed light on therapeutic mechanisms of standard antipanic treatments, such as SSRIs.[24] In addition, ascertainment of the utility of plasma ORX as a biomarker of illness state and clinical improvement could immediately impact practice.

Furthermore, such a finding may well have future transdiagnostic implications with regard to the pathogenesis of panic symptoms that occur in a variety of psychiatric syndromes (e.g., other anxiety disorders, trauma-related disorders) and in medical disorders with panic as a component, such as chronic obstructive pulmonary disease and chronic asthma.

Public Health Impact of the Project

PD is a common psychiatric condition with a lifetime prevalence of up to 4% in the general community.[25,26] It is frequently a chronic relapsing or persistent illness, even after standard treatments.[27] It can profoundly affect functioning and quality of life; is often associated with serious co-morbidity (particularly with MDD, other anxiety disorders, and substance abuse);[28,29] and predisposes to suicide attempts.[30,31] It is clear that new, more potent antipanic treatments are urgently needed.

STUDY PROCEDURES (see Table 2)

Rationale for the Proposed Design

Our design adopts a “fast-fail” experimental medicine approach to testing our treatment hypothesis, thereby avoiding an expensive weeks-long clinical trial design with the prospect of exposing patients to an extended period of placebo and adverse events (e.g., fatigue, somnolence). A single, standard 10 mg dose of suvorexant has documented CNS/soporific effects,[32,33] is highly bioavailable, and

takes 120 minutes to achieve peak plasma concentration. Though suvorexant is a mixed ORX1/2 antagonist (the ORX1 receptor has generally been more linked to fear behaviors),[34,35] it binds to both ORX receptors with nanomolar affinity and high selectivity.[14] To date, a major challenge for ORX studies of anxiety and mood disorders has been to establish the validity of CSF and plasma ORX as indices of brain ORX function. The 2014 market release of suvorexant now permits rigorous cross-validation of these measures.

As mentioned above, we selected the CO_2 test paradigm because it is an extremely well-validated, safe, and easy-to-administer test, and it closely reproduces naturalistic panic.[18,20,36] In addition, ORX neurons are known to be chemosensitive,[37] and animal work has recently demonstrated ORX neuronal excitability in parallel with CO_2-evoked anxiety.[35]

Recruitment and Clinical Care

Patient recruitment, the screening visit (study visit 1), and the follow-up (study exit) visit will be conducted at the UCSF Fresno Clinical Research Center (CRC). The study intervention visit (study visit 2) will be conducted in an exam room in East Medical Plaza (University Neurology Associates). Patients will be referred from a variety of sources including local physicians, psychiatrists and psychologists; paid advertisements appearing in local newspapers; free advertisements in the local Craigslist "volunteers" section; and from internal medical center clinics (the UPA and ACC outpatient clinics).

Patient Selection and Preliminary Evaluation (Study Visit 1—Screening Visit)

Patients between the ages of 18 and 65 years will be admitted to the study after they have given their written informed consent to participate. Study participants will also be given the choice whether to give their additional written informed consent to provide a blood sample for future DNA analysis.

Patients will undergo a complete medical and psychiatric evaluation by a study doctor assisted by the appropriate study/hospital staff (see Table 2), including history taking, physical examination, diagnostic interview, electrocardiogram, laboratory tests (comprehensive metabolic panel, CBC, free T4, urine pregnancy test, urinalysis), urine toxicology screen, and urine pregnancy test in women of child-bearing potential.

Inclusion criteria: To participate in the study, patients must meet the following inclusion criteria:

- Men and women between the ages of 18 and 65 years are eligible for inclusion in this study.

- They must be in stable physical health as determined by a medical evaluation (see Table 2), including physical examination, electrocardiogram, laboratory findings (comprehensive metabolic panel, CBC, free T4, urine pregnancy test, urinalysis), urine toxicology screen, and a negative urine pregnancy test in women of child-bearing potential.

- They must satisfy the new DSM-5 clinical criteria for a current principal diagnosis of PD[38] as confirmed by a semi-structured, diagnostic interview, the MINI,[39] administered by the PI, Dr. Goddard.

- Since clinical depression (MDD) is associated with CSF ORX abnormalities,[40] only patients with a current PD without MDD will be enrolled. They will also be required to have a current Montgomery-Asberg Depression Rating Scale (MADRS) total score <12.[41]

- They will be off all regular psychiatric medications and avoid drinking grapefruit juice for at least 2 weeks prior to the 35% CO_2 test.

- They must not be pregnant or breastfeeding a baby; and women of childbearing potential must be using birth control while on this study.

Exclusion criteria: The following is a list of the exclusion criteria in reference to this study:

- any history of a psychotic disorder, bipolar disorder, MDD, depression NOS, obsessive compulsive disorder, an eating disorder, post-traumatic stress disorder, or generalized anxiety disorder

- medical conditions for which suvorexant could be contraindicated, such as narcolepsy

- any other sleep disorder[42]

- a substance use disorder, as defined by the DSM-5, within 6 months of the screening visit

- ongoing use of psychiatric medications in the 2 weeks prior to the 35% CO_2 test

- current use of certain drugs,[43] including

    - strong CYP3A inhibitors (such as ketoconazole, itraconazole, posaconazole, clarithromycin, nefazodone, ritonavir, saquinavir, nelfinavir, indinavir, boceprevir, telaprevir, telithromycin, and conivaptan);
    - moderate CYP3A inhibitors (such as amprenavir, aprepitant, atazanavir, ciprofloxacin, diltiazem, erythromycin, fluconazole, fosamprenavir, imatinib, verapamil);
    - strong CYP3A inducers (such as rifampin, carbamazepine and phenytoin);
    - digoxin

- history of any neurological disorder affecting the CNS

- history of uncontrolled or serious medical illness

- a history of hypersensitivity or allergy to suvorexant

- pregnancy or lactation status, or unwillingness to use birth control while on this study, for women of child-bearing potential

- compromised lung function (e.g., COPD, emphysema, idiopathic pulmonary fibrosis, lung cancer)
- inability to fast the required amount of time prior to study visit 2
- a positive test for cannabinoids, opiates, benzodiazepines, amphetamines, cocaine and metabolites
- out-of-range lab values
- an abnormal EKG
- a score > 12 on the Montgomery-Asberg Depression Rating Scale (MADRS)
- inability or unwillingness to avoid drinking grapefruit juice for two weeks prior to the 35% CO2 challenge test
- a history of sudden onset of muscle weakness (cataplexy)

  Notes regarding the urine toxicology tests and benzodiazepine screening: The purpose of the urine screening is twofold: (1) study validity (patients are excluded if the screening reveals use of certain substances, when by history patients have denied taking); (2) patient safety (the sedative effects of the study drug and a controlled substance, such as a benzodiazepine or pain medicine, could be additive). Substances screened for include cannabanoids, opiates, benzodiazepines, amphetamines, cocaine, and metabolites.

If the PI believes that any subjects are a "borderline case" for inclusion / exclusion, he will discuss the issue with the Safety Monitor or the patient's primary care doctor, as appropriate.

TABLE 2: STUDY PLAN & SCHEDULE OF ACTIVITIES

Visit	**1** Screening (2 hr)	**2** CO_2 Test visit (3.5 hrs)	**3** Follow-up (exit) visit (0.5 hr)
Days/Weeks		About 1 week after visit 1	About 1 week after visit 2
Informed consent – orexin study	X		

Informed consent – DNA sample	X		
Psych Hx/Diagnosis Interview/MINI	X		
Inclusion/exclusion criteria	X		
Medical history	X		
Physical examination	X		
Vital signs	X	X (pre-, during, and post-CO_2)	
ECG	X		
Comprehensive metabolic panel; CBC w/diff; free T4	X		
Urine pregnancy test (females)	X		
Urinalysis	X		
Urine toxicology screen	X		
Urine benzo screen		X	
SUVOREXANT 10MG/PLACEBO.		X (120 mins pre CO_2)	
OREXIN LEVELS		X (pre-, during & post- CO_2 challenge)	
DNA sample (optional: obtained only if additional consent form is signed)		X	
PDSS/CGI clinical scales		X (completed by a study doctor pre-CO_2)	
Visual Analog Scales (Anxiety) and Panic Symptoms Scale (PSS)		X (completed by subjects pre-, during, and post-CO_2)	
Interview with MD to verify return to baseline			X

Referral to appropriate doctor to treat panic (if desired)			X

35% CO_2 Inhalation Test Procedures (Study Visit 2, see Table 3)

After the screening visit (visit 1), patients will be scheduled in about 1 week for the CO_2 challenge test (study visit 2), which will take place an exam room in East Medical Plaza (University Neurology Associates).

Upon arrival for study visit 2, each participant will receive a consecutive study code. The master list of study codes will be linked with a random assignment of suvorexant and placebo. The randomization schedule will be generated by Paul Mills, PhD, using a computer program. The pharmacy will maintain an independent list of the study codes and randomization assignments (to either 10mg suvorexant or placebo).

The following instruments will be used by the PI or sub-I to assess baseline PD clinical severity just prior to the CO_2 test:

- the Clinician's Global Impressions of severity of illness (CGI-S)[44]
- the Panic Disorder Severity Scale (PDSS)[45]

The following instruments will be administered to subjects at baseline and serially throughout the test:

- a change-sensitive, patient-rated, Vertical Analog Scale (VAS) mood ratings (5 mood states including fear, nervousness, anxiety, rated 0-100)[46]
- a Patient Panic Symptom scale (PSS; 27 items, each rated 1-4)[46]

The PI or sub-I will be present when the subject takes the study drug or placebo (-120 minutes; see Table 3). At that time, and until the CO_2 inhalation test is about to begin (-120 mins to 0 mins), the Research Coordinator will be present in the room. From the time of the CO_2 inhalation test (0 mins) until the patient leaves accompanied by a friend or family member (+60 mins), both the Research Coordinator and the PI or sub-I will be present in the room.

Table 3 CO_2 Inhalation Test Procedures

Timing	*Activity/Procedural Step*	*Measures*
-130 mins	Patient welcomed to the CRC. Taken to the testing room. Baseline behavioral ratings and vital signs obtained. 7 cc blood drawn (ORX)	PDSS, CGI-S; Vital Signs (VS); ORX
-120mins	Administer single oral dose of suvorexant 10 mg or identical-appearing placebo with water.	
-10 mins	Obtain pre-CO_2 test baseline behavioral and physiological (vitals) measures.	VAS (5 items), PSS; VS

-5 mins	Plastic mask attached, and patients on room air for 5 minutes. 7cc blood drawn (ORX)	ORX level
0 mins	Blindly switch to 35% CO_2 / 65% O_2 gas mixture inhalation for 60 seconds.	
+1 mins	Monitoring for panic/anxiety symptoms, ORX	VAS, PSS, VS, ORX
+5 mins	Monitoring for panic/anxiety symptoms	VAS, PSS, VS
+15 mins	Monitoring for panic/anxiety symptoms, ORX	VAS,PSS,ORX, VS
+30 mins	7 cc blood drawn (ORX)	ORX
+60 mins	Monitoring for panic/anxiety symptoms. If patient stable discharge from the CRC, accompanied by a family member, friend, or caregiver.	VAS,PSS VS

ORX and DNA blood samples: Blood samples for ORX ELISA analysis will be collected, prepared and processed by the study coordinators. The samples will be collected into 7 ml Vacutainer tubes (#VT-6450) which contain EDTA (lavender top). The tubes will be shaken gently several times immediately after collection to promote anti-coagulation. Blood will then be transferred to centrifuge tubes and centrifuged at 1,000 x g for 15 minutes at 4°C. 1 mL plasma samples will be removed, and stored in a -80°C freezer located in the CRC.[47] From those subjects who have provided their (additional informed consent to allow a blood sample to be collected for future DNA analysis, a sample of this centrifuged blood (after the plasma has been removed) will be banked in a -80°C freezer located in the CRC for future candidate gene/DNA analysis. Genes of interest with respect to ORX metabolism are the preproORX gene and genes that code for the receptors, ORX1 and ORX2.

Suvorexant/Placebo Medication, and Rescue Medication

The pharmacotherapy will be supervised by the PI, Dr. Goddard, who is very experienced in the treatment of patients participating in clinical trials.

Suvorexant: 10mg tablets will be used. Patients who receive suvorexant vs placebo will receive a total of one 10mg dose. Tablets will be formulated into capsules by Script Life Pharmacy, Clovis, CA, in order to be identical in appearance to placebo. It is acceptable to CRMC pharmacy that Script Life Pharmacy compound the study drug.

Matching placebo capsules: These will be prepared by Script Life Pharmacy, Clovis, CA, to appear identical to the suvorexant caps mentioned above. It is acceptable to CRMC pharmacy that Script Life Pharmacy compound the placebo.

Lorazepam: 0.5 tablets will be used. (As explained below, based on the PI's clinical interaction with and observations of the patient, lorazepam 0.5 mg po will be available for use as a rescue medication if patients have a pronounced or prolonged CO2 panic response following CO2 administration.) These will be purchased from Script Life Pharmacy, Clovis, CA. It is acceptable to CRMC pharmacy that Script Life Pharmacy provide the lorazepam.

Drug safety data: A summary of drug safety data for suvorexant can be found in the FDA-approved package insert.[43] In non-elderly adults treated up to a month on a dose of 10 mg, the most common adverse reaction reported was somnolence, 2%, compared to <1% for placebo.

Storage and dispensing: All investigation/study products will be stored at and dispensed by the CRMC Pharmacy.

Accountability: The study drug, placebos, and lorazepam must only be used as directed in the protocol. Records of overall dispensing will be maintained separately from the case report forms recording the medication dispensed to each patient. The PI and CRMC pharmacist will make sure that unused or expired study medications are destroyed in an appropriate manner. Per the CRMC Pharmacy, unused medications are to be returned to the Pharmacy and their destruction will be verified by 2 pharmacists.

Methods for ensuring blinding: The CRC study coordinator will interact with the CRMC pharmacist to obtain appropriate medication for each patient, according to the randomization schedule. Two sets of study blinds/codes will be kept in sealed envelopes inside a small box in a locked cabinet: one within the UCSF-F CRC, and one within the UPA clinic.

Methods for unblinding the study: The treatment code must not be broken except in medical emergencies when the appropriate management of the subject necessitates knowledge of the treatment randomization. In the event of such a patient emergency, the medication blind will be revealed to the appropriate emergency medical staff by the coordinator or PI. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented.

Follow-up Contacts

After subjects complete their participation in study visit 2, and as an extra measure to ensure their ongoing well-being, we will

- Make a follow-up phone call to subjects the day after study visit 2, to ask them how they are doing, and to verbally verify that a family member stayed with them the appropriate amount of time after study visit 2.
- Complete one follow-up visit with each subject and the PI or a sub-I at the CRC to ensure that subjects have returned to baseline.

From time of enrollment through return to baseline, subjects will be enrolled in the study approximately two weeks.

Because subjects' participation in the study does not provide any treatment for their condition, after subjects complete their participation in the study, they will be offered the opportunity to receive up to four treatment visits from the PI or a sub-I for their panic disorder, after which they will be referred back to their primary care provider. If subjects choose not to accept the treatment visits, they will be referred back to their primary care provider after the follow-up visit (described above).

Adverse Events/Safety Monitoring

Side effects will be assessed clinically at the patient's study visit by the PI, via serial vital sign monitoring and behavioral monitoring. The PI will present a 6-monthly report of study progress to the UCSF-Fresno/CRMC IRB. This will facilitate recognition of any patterns of adverse events, and provide feedback on managing these better as the study progresses.

Based on the PI's clinical interaction with and observations of the patient, lorazepam 0.5 mg po will be available for use as a rescue medication if patients have a pronounced or prolonged CO_2 panic response following CO_2 administration. There is evidence in psychiatric patients that acute benzodiazepine ingestion/overdose may not greatly alter CSF ORX levels,[40] and hence plasma ORX data obtained if patients have had rescue medication may still have validity.

Because of the small risk of somnolence in our study subjects, we will require that subjects not drive home after study visit 2. The day of study visit 2, before any drug is administered, investigators will ensure that the subjects have a plan to be accompanied by a family member, friend or caregiver, and that this individual will be in the subjects' company for the remainder of 7 hours from the time they took the drug. This individual must ensure their transport home and report any adverse events to the investigator at (559) 320-0580.

In the unlikely event of an adverse reaction requiring medical or psychiatric care, patient coverage will be available 24/7 through the UCSF-Fresno psychiatry on-call schedule, who will be informed that the PI is available to be contacted via cell phone or email at any time. On-call activities and interaction are not charged in Psychiatry. In the unlikely event of additional ED or urgent care treatment, this will be covered by UCSF, depending on a number of factors.

All adverse events that qualify as Unanticipated Problems will be reported to the IRB within 10 days; any subject deaths will be reported to the IRB within 24 hours of a member of the study team learning about the death.

The Safety Monitor

The PI will present this project to Wee Kiat (David) Lee, MD, the Safety Monitor, for an initial review shortly after IRB approval. Thereafter, 3 monthly monitoring reviews will be conducted with the PI presenting a brief synopsis of adverse events, reasons for drop-out, and study progress. If there is a serious adverse event (e.g. patient hospitalized for medical or psychiatric reasons during the protocol, or a patient death), an emergency meeting with the Safety Monitor will be convened.

Data Management and Statistical Considerations

The study coordinators, working together with the study research manager, will be responsible for overseeing data collection and accuracy of record keeping. All of the hard copy research data will be kept in locked file cabinets in the study manager's office. Only the PI, sub-Is study coordinator will have access to these files, ensuring the security of the hard copy records. Each subject will be given a unique alphanumeric code and this will serve as the only connection between the locked files, the hard-copy forms, and the electronic database. All forms to be used in the study will only contain this alphanumeric code to protect personal health information and maintain HIPAA compliance. Additionally, other procedures to ensure confidentiality will follow the regulations and policies of UCSF Fresno and CMC.

Data will be collected on hard-copy forms and then verified by the study coordinator, who will be trained to search for potential errors. Any questionable or illegible entries will be brought to the PI's attention in a regularly recurring research meeting (used to monitor recruitment, data collection, and protocol compliance). A full review of new data acquired in the previous week will be performed and those subjects passing the final verification will have their forms placed into the secure file cabinets. The forms not passing verification will be addressed immediately.

The electronic database used to house these data until analyses are needed will be a Microsoft Access database created uniquely for this project and kept on the UCSF server. The database will be password protected and only certain users will be given access to the folder containing the database. All data will be de-identified and the key will be kept in a locked drawer in the study manager's office. This will protect the electronic data against any unauthorized persons from entering the dataset and jeopardizing the integrity of the data. All versions of the forms in the Microsoft Access database will have interfaces nearly identical to the hard copy forms for ease and efficiency of entry. This will substantially limit the number of typographical errors by alerting data entry personnel when an entry does not match the list of possible entries dictated by the form. As this project matures into the data analysis stage, data will be queried and exported to statistical software for data analysis.

All data analyses will be performed using SPSS version 22. Continuous variables will be summarized separately in the two PD patient groups using descriptive statistics, including mean, SD, minimum and maximum values. Categorical variables (e.g., presence/absence of a CO_2-panic episode) will be summarized using frequency counts and percentages. Baseline demographic data will be compared between the two groups. Dichotomous and ordinal variables will be examined using Fisher's exact tests, and continuous measures (e.g., peak change from baseline in CO_2-evoked VAS anxiety, and peak change from baseline in plasma ORX) with Wilcoxon rank sum tests. Effect sizes will be generated to facilitate planning of a definitive study.

Since our current pilot data (see p. 3) are so limited, the data generated through this study will enable us to estimate power requirements for a larger study, and to fine-tune the protocol. A speculative power estimate for the larger study, based on our current plasma ORX/CO_2 findings, is as follows:

If we assume a mean±SD peak change value of 50±25 pg/ml for the PD/placebo group, and 30±25 pg/ml for the PD/suvorexant group, we would have 80% power to detect this difference, with α=0.05, utilizing a 2-tailed Students t-test, with 25 patients in each group.

The pilot study timeline will be as follows: 3 months to start up and obtain IRB approval; an 8-month enrollment period, recruiting 1-2 patients per month; and a final month for analysis, poster development, and development of a grant application to fund a definitive study of the research questions being addressed by this pilot work.

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