MEMORANDUM

To: James Eisenach
Anesthesia

From: Gregory Kucera, Chair, IRB # 2
Institutional Review Board

Date: 1/25/2022

Subject: Human Protocol: IRB00079629
A Dose Ranging Study for a Pharmacodynamic Model of Oxytocin for Peripheral Analgesic Effects

Study Documents:
Protocol Version: Protocol_A Dose Ranging Study for a PD Model of Oxytocin Infusion__12_7_2021; Informed Consent Version: ICF_A Dose Ranging Study for a PD Model of Oxytocin Infusion__12_7_2021; Investigator's Brochure: FDA_Delivery Receipt_protocol_12_8_2021, FDA_IND_Initial Submission, INVESTIGATOR BROCHURE_IV_IM_Oxytocin_9_10_2020; Other Documents: Appendix_1_Grid_Protocol_2A, Appendix_2_Data Collection Form_Protocol_2, INVESTIGATOR BROCHURE_IV_IM_Oxytocin_9_10_2020, Pitocin package insert

The Institutional Review Board (IRB) has approved the above-named protocol and study documents, after review at a convened meeting on 1/25/2022. A submission requesting renewal together with a summary progress report must be submitted to the Board at least one month prior to 1/24/2023.

Approved; JIT submission; If funding is awarded, please submit an application amendment to address/update the following within the application and consent form prior to initiation of study activities:

Clinical Trials Page: Section 6.0; Please insert the NCT#, "NCT05179421."
Safety, Confidentiality, Privacy page: Per protocol, please update section 4.0 to select, "DSMB Chartered by PI." and remove, "Safety monitor(s), medical monitor(s) or other independent individual(s)."
Consent form page: Please update risks within the consent form to include burning and/or redness as a possible side effect.

This submission has met the requirements of the 2019 Common Rule.

Federal regulations and Board policy require that you promptly report to the Board for review/approval:

- Proposed changes in the research activity (e.g., protocol amendments; consent form revision; advertisements). Changes may not be initiated without IRB review and approval, unless necessary to eliminate an immediate hazard to subjects.

- Serious adverse events and unanticipated problems involving risks must be reported to the Board, institutional officials, FDA, sponsor and other regulatory agencies as required by the protocol, local policy and state or federal regulation.

Please provide a final report to the Board when the project is completed and Board approval can be terminated.

IRB registration numbers are IRB00000212, IRB00002432, IRB00002433, IRB00002434, IRB00008492, IRB00008493, IRB00008494, and IRB00008495.
WFSM IRB has been continually fully accredited by the Association for the Accreditation of Human Research Protection Programs (AAHRPP) since 2011.

Medical Center Boulevard, Winston-Salem, NC 27157-1023 (336) 716-4542 / fax (336) 716-4480

Dose ranging study of intravenous oxytocin for analgesia to heat pain

Principal Investigator: James C. Eisenach, M.D., Co-investigator(s): Douglas Ririe, M.D.

Sponsor or funding source: NIH

Background, Rationale and Context

History of recent oxytocin research at Wake Forest

The Pain Mechanisms Laboratory began work on an NIH program project grant (P01) proposal in 2019 focusing on basic research on oxytocin mechanisms of analgesic action in animals and clinical trials to establish oxytocin analgesic efficacy in humans. We received IRB approval for 3 protocols involving intravenous (IV) or intramuscular (IM) oxytocin under a waiver of IND requirement from the FDA to acquire preliminary data necessary for this NIH submission:

IRB number	Approval date	Title
56413	4/29/2019	PK Sampling after IV Oxytocin Injection in Healthy Subjects
56660	4/29/2019	Effects of IM Oxytocin in UV Burn
60838	10/1/2019	Effects of IM Oxytocin on Pupil Diameter and HRV

The first study determined a preliminary model for the pharmacokinetics (PK) of oxytocin in plasma after IV administration using the state-of-the-art liquid chromatograph / mass spectroscopy (LC/MS) method and also examined pain report to 5-minute heating of the skin after oxytocin administration. Oxytocin concentrations were best fit to a two compartment PK model and the time course of oxytocin to reduce pain from the heat stimulus was used to create a pharmacokinetic/pharmacodynamic (PK/PD) model. The second study was intended to test the analgesic effects of IM oxytocin after controlled UV-B burn to a small area of skin as a model of peripheral nerve hypersensitivity. Despite generation of erythema, the burn produced minimal hypersensitivity on the following day (the day of study), so we were unable to test oxytocin's effect. The third study demonstrated that IM oxytocin did not affect average pupil diameter or heart rate variability (HRV) over time, but did reduce slow frequency oscillations in pupil diameter, a phenomenon termed hippus, that reflects parasympathetic tone to the iris. The time course of this action of oxytocin was coupled with the PK data to create a PK/PD model for central nervous system action of IV administered oxytocin. These data were central to the P01 grant submission in January, 2020.

In anticipation of NIH requirement for IRB approval at the time of NIH Council meeting in September, 2020, six protocols were submitted to the Wake Forest School of Medicine IRB. These were approved pending FDA approval of an IND. That IND was approved in November, 2020, leading to full IRB approval.

IRB number	Approval date	Title
65787	6/15/2020	IV Oxytocin PK: Healthy Subjects and Advanced Knee Arthritis
66130	6/18/2020	Estimate Serum Oxytocin Pharmacodynamic Range in the

		Periphery
66136	7/6/2020	Generate a PD model of oxytocin for peripheral analgesic effects
66138	6/10/2020	Validate a PD model of oxytocin for peripheral analgesic effects
66443	7/16/2020	Apply PD Model of Peripheral OXT Action to a Multimodal Stimulus
66475	7/9/2020	Generate Test Reliability of PD Model of OXT on Pupil Hippus

The P01 application received a good impact score of 37 but was not recommended for funding by Council. In response to reviewer concerns, we initiated IRB00066136 to replicate the initial observations of oxytocin induced pain relief to a sustained heat stimulus. This was successful and the preliminary PK/PD model from the first study fit well the time course and degree of analgesia in this second study.

The P01 was extensively revised and resubmitted in January, 2021. This included revisions to each of the 6 proposed studies with IRB approval. The grant resubmission received an impact score of 20, was recommended by NIH Council for funding 3 months ago, and NIH Program in late November informed us that they anticipate requesting last minute documents in preparation for funding shortly.

This IRB application is for a revision to IRB00066130 (which has not been initiated) to estimate the IV oxytocin dose-response for analgesia to 5 min heating of the skin. We considered amending the approved protocol, but as noted in the table below, the study design was altered in so many fundamental ways in response to the NIH review that we felt a replacement was more appropriate.

Factor	IRB00066130	This study
Population	Healthy volunteers and those with advanced knee osteoarthritis	Healthy volunteers age 18-75, stratified by age
Sample size	20	24
Oxytocin dosing	Stepped 15 min infusions to target escalating plasma oxytocin concentrations	Constant rate 10 min infusions of placebo or 3 different oxytocin doses
Total oxytocin dose	41 mcg	1.3 or 7.3 mcg
Blinded	No	Yes
Blood sampling	Yes	No

Rationale for current application

Both preclinical and clinical evidence suggests that oxytocin may serve as a novel pain therapeutic and potential disease-modifying agent to prevent the transition from acute to chronic pain. This follows from observations that pain resolves quicker in women after cesarean delivery than other surgeries [1], and that hypersensitivity resolves quicker in rodents when neuropathic injury is performed after delivery, an effect blocked by inhibition of oxytocin action [2, 3]. Oxytocin may act peripherally to affect pain after injury. Since chronic pain is usually reduced acutely by peripheral nerve block, peripheral input is necessary, but most research assumes that input is normal and pain reflects ongoing central sensitization. We and others challenge

these ideas, showing that touch sensitive nerve fibers are desensitized after injury whereas fast conducting pain nerve fibers are sensitized [4]. In rodents, oxytocin acutely moves these two abnormal signals after injury towards normal [5], suggesting a disease-modifying potential of oxytocin as a 'vaccine' against chronic pain after injury.

Oxytocin itself is the only clinically available tool for translational studies in many areas – neuroprotection, anxiety, sleep, social behaviors, addiction, and pain. Most rodent and human studies of oxytocin are small, lack strong scientific rigor, and only half of the clinical studies examining pain demonstrate efficacy. Thus, we have minimal ability to understand oxytocin effects across species. A critical problem is the complete lack of fundamental pharmacologic characterization for oxytocin, which is used clinically in obstetrics by titration-to-effect. There are few PK and no PK/PD modeling studies of oxytocin in the literature, and the PK studies use RIA and ELISA assays which are unreliable [6].

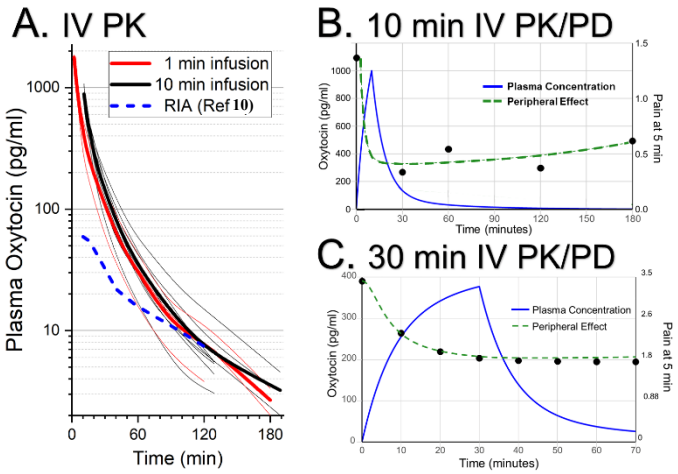

Figure 1. **A)** plasma oxytocin concentrations with LC/MS (red/black lines) compared to plasma by RIA (blue lines. **B)** modeled plasma oxytocin concentrations (blue line) and observed (circles) and modeled (green dash) pain scores after 10 min infusion of 17 µg oxytocin. **C)** as in B) but after 30 min infusion of 14 µg oxytocin

Under IRB00056413 we measured plasma oxytocin concentrations after IV administration, using an LC/MS assay [7]. **Figure 1A** shows individual (thin lines) and group (thick lines) after oxytocin, 10 IU (17 µg). The first 3 individuals received a 1 min infusion, which was accompanied with headache and sensation of flushing (red lines) and the remaining 8 volunteers received a 10 min infusion with 5 of 8 subjects experiencing symptoms. These data were well fit to a 2-compartment PK model using NONMEM. Note the large discrepancy between these data and a recent report [8] in which the same dose of oxytocin was infused over 10 min and plasma oxytocin measured by radioimmunoassay (RIA).

To assess peripheral effects, we used pain report to 5 minute exposure to a 45°C stimulus, since our studies in rodents shows that prolonged noxious heat sensitizes and increases activity the fast conducting pain fibers which are sensitive to oxytocin [9]. **Figure 1B** shows the modeled plasma oxytocin concentrations in the 11 subjects in **Figure 1A** and pain report to a 5 min noxious heat exposure. The data were nicely fit to a PK/PD model yielding parameters of 25 min to peak effect, an EC50 of 10 pg/ml, and steepness of concentration response (γ) of 1 (green dashed lines). The goal of this PK study was to determine oxytocin PK and to explore a potential effect on pain from skin heating. Under IRB00066136 we examined the time course of analgesia to skin heating by slower infusion of IV oxytocin (0.47 µg/min for 30 min for a total dose 14 µg) in 10 volunteers. There were no adverse events with this dosing regimen, and the PK/PD model from the first study accurately predicted the time course of onset of analgesia during infusion and maintenance of analgesia after the infusion was stopped (**Figure 1C**).

The primary objective of the current IRB application is to determine the dose response of IV oxytocin for analgesia to experimental heat pain at the time of anticipated maximum effect.

The proposed study design will advance the preliminary data by use of a placebo controlled, partially randomized dose response with each subject being tested on two separate study days and receiving on each study day two 10-min IV infusions of study drug separated by one hour (**Figure 2A**). As shown in this figure, the preliminary PK/PD model predicts that infusions of 0.3, 1.3, and 7 µg in these paradigms will result in analgesic effects of approximately 20%, 50%, and 85% of the maximum. The PK/PD model further predicts a relatively stable drug effect with this infusion paradigm from 10 to 50 min after the end of the infusion.

These infusion times and oxytocin dosages were also chosen to minimize adverse events observed in our previous studies. In the original PK study (IRB00056413) adverse events were observed in 5 of 8 individuals who received a 10 min infusion of 17 µg oxytocin. Adverse events were facial flushing and sensation of hear pounding despite no change in heart rate or blood pressure, similar to adverse events commonly observed with rapid IV infusion of oxytocin at cesarean delivery to rapidly contract the uterus after delivery of the fetus. Adverse events were not observed below plasma oxytocin concentrations of 400 pg/ml, and in the second study (IRB00066136) in which we infused oxytocin to a modeled concentration of 400 pg/ml, no adverse events were observed. As shown in **Figure 2B**, plasma oxytocin concentrations with the largest oxytocin infusion are modeled to not exceed 400 pg/ml, and we therefore anticipate few if any of these adverse events.

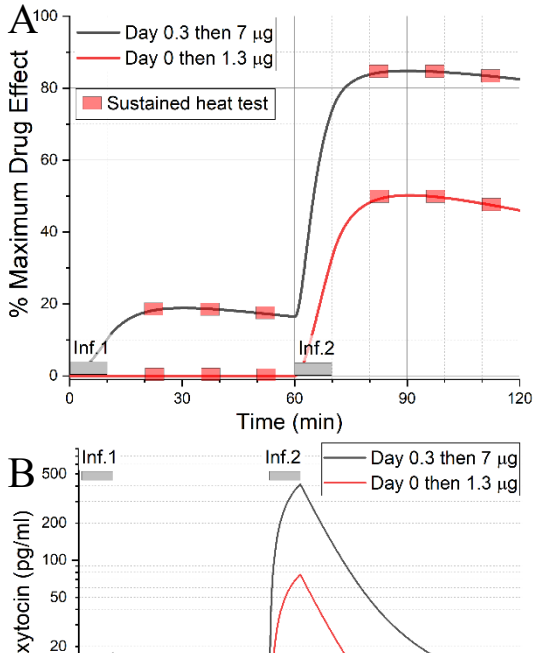

Figure 2. A) predicted drug effect over time after two 10-min IV infusions (Inf. 1 and 2) separated by one hour on 2 study days, one with 0.3, then 7 µg and the other with 0, then 1.3 µg. Time of heat pain testing shown in red squares. **B)** predicted oxytocin plasma concentrations following the infusions in **A)**

Objectives

Main objective Determine dose-response of IV oxytocin infusions to reduce pain from an experimental heat stimulus

Additional Objectives

1. Test for effects of subject sex or age on oxytocin effect
2. Refine the PK/PD model for oxytocin analgesic action based on time course of observations

Methods and Measures

Equal numbers of adult men and women will be recruited. Sample size will be divided into 3 groups; young (18-39-year-old) middle (40 – 59-year-old) and older (60 – 75-year-old). We anticipate that approximately 20% of subjects will be in the elderly group, 35% will be in the middle age group, and 45% will be in the young group.

Subjects will report to the Pain Clinical Research Unit (CRU) or the CTSI CRU for three visits

We will abandon an individual participant's study and schedule another participant should the participant report minimal pain to the heat stimulus on the training visit day or should we be unable to successfully place the intravenous catheter.

STUDY DESIGN

This is a blinded, randomized, controlled study of IV infusion of placebo and 3 doses of oxytocin. In this study, healthy individuals will come to the Piedmont Plaza 2 Pain Clinical Research unit (CRU) or the CTSI CRU for a training visit and then to the CTSI CRU for two other study days, separated by at least 72 hr. During the training day they will report pain on a 0 (no pain) to 10 (worst imaginable pain) scale each minute during a 5 min heating of the skin of the lateral calf to a temperature (45°C) using an FDA approved, commercially available device (Medoc, TSA II). If pain score is less than 2 at the end of this stimulus, the test will be repeated with the probe set to 46ºC. If pain score is less than 2 on that occasion, the test will be repeated with the probe set to 47ºC. If pain score is less than 2 on that occasion the individual will not go on to further study and will be reimbursed for the training day only. On the other two visits, subjects will receive on one visit IV saline followed in one hour by IV oxytocin, 1.3 µg and on the other visit they will receive IV oxytocin, 0.3 µg followed in one hour by IV oxytocin, 7 µg. Infusions will be given over 10 minutes. The order of these two dosing regimens will be randomized and double blind, with solutions released from the research pharmacy. As a result, each subject will receive oxytocin doses of 0, 0.3, 1.3, and 7 µg in this double blind design. Pain report each minute and at the end of a 5-min heating will be recorded before the first infusion and then with the 5-min heat test ending at 15, 30, and 45 min after the ending of the infusion. The probe temperature used for heating will be that determined during the training day. Immediately after the 60 min pain report the second infusion will be given and pain intensity at the end of 5-min of heating recorded at 15, 30, and 45 min thereafter. The study will then end.

Phase II trials

Setting

The training visit will occur at either the Pain CRU at Piedmont Plaza 2 or the CTSI CRU. Drug study visits will occur on the main campus at CTSI CRU.

Subjects selection criteria

The study will enroll healthy subjects.

Inclusion Criteria

1. Male or female ≥ 18 and < 75 years of age, Body Mass Index (BMI) <40.
2. Generally in good health as determined by the Principal Investigator based on prior medical history, American Society of Anesthesiologists physical status 1, 2, or 3
3. Fully vaccinated against COVID-19
4. For healthy volunteers, normal blood pressure (systolic 90-140 mmHg; diastolic 50-90 mmHg) resting heart rate 45-100 beats per minute) without medication. For those with hypertension, blood pressure controlled with anti-hypertensive medication and with a resting heart rate 45-100 beats per minute.
5. Female subjects of child-bearing potential and those < 1 year post-menopausal, must be practicing highly effective methods of birth control such as hormonal

methods (e.g., combined oral, implantable, injectable, or transdermal contraceptives), double barrier methods (e.g., condoms, sponge, diaphragm, or vaginal ring plus spermicidal jellies or cream), or total abstinence from heterosexual intercourse for a minimum of 1 full cycle before study drug administration.

Exclusion Criteria

1. Hypersensitivity, allergy, or significant reaction to any ingredient of Pitocin®
2. Any disease, diagnosis, or condition (medical or surgical) that, in the opinion of the Principal Investigator, would place the subject at increased risk (active gynecologic disease in which increased tone would be detrimental e.g., uterine fibroids with ongoing bleeding), compromise the subject's compliance with study procedures, or compromise the quality of the data
3. Women who are pregnant (positive result for serum pregnancy test at screening visit), women who are currently nursing or lactating, women that have been pregnant within 2 years
4. Subjects with neuropathy, chronic pain, diabetes mellitus, or taking benzodiazepines or pain medications on a daily basis.
5. Subjects with current or history of ventricular tachycardia, atrial fibrillation or prolonged QT interval.
6. Subjects with past or current history of hyponatremia or at risk for hyponatremia; anyone taking thiazide diuretics, loop diuretics, combination diuretics, lithium, carbamazepine, enalapril, Ramipril, celecoxib, temazepam, gliclazide, glimepiride, glibenclamide, glipizide, omeprazole, pantoprazole, desmopressin, SSRI's, MAOI, or the recreational drug ecstasy.
7. Subjects with a known latex allergy.

Sample Size

The projected sample size is 24 subjects. Our current estimates indicate that the confidence interval for studies of 21 individuals with pain intensity ratings will determine the pharmacodynamic parameters with a corrected confidence range < 20%. A corrected confidence range < 20% implies that mixed effect analysis will distinguish covariate effects in the range of 40% in a study with 21 or more subjects. This is the range at which the covariate effect may be clinically consequential. In practice, mixed-effects analysis with NONMEM is often sensitive to covariate effects smaller than predicted by preliminary analyses. The sample size is increased to 24 to provide stratification by age (3 strata), weight (2 strata), and sex. The crossover design permits accurate determination of dose linearity despite expected high inter-subject variability in pain ratings.

Interventions and Interactions

At all study visits temperature will be measured and participants queried regarding recent history of symptoms commonly associated with COVID-19 or recent close contacts with anyone with a positive COVID-19 test.

Study Visit 1:

The participant will report to the Pain CRU or the Wake Forest CTSI CRU. The participant will review and sign the Informed Consent. After informed consent is adequately

obtained, a detailed medical history will be obtained from the participant, female participants will have a urine pregnancy test performed to determine pregnancy status. Vital signs to include blood pressure (BP), heart rate (HR), respiratory rate (Resp) and oxyhemoglobin saturation by pulse oximetry (POX) will be measured and recorded along with height and weight.

Once eligibility has been determined the participant will be trained in study procedures. This will include pain ratings to brief (5 sec) heat stimuli on the lateral calf to 39, 41, 43, 45, 47, and 49ºC and then a pain intensity rating each minute for 5 mins during heating to 45ºC. If the pain report to 45ºC is < 2 at the end of the 5 min, a second trial using 46ºC will be done, and if that also results in pain intensity < 2, a third trial with 47ºC will be performed. Subjects who have pain intensity < 1 with the 47ºC 5-min stimulus will not be further studied. The duration for visit 2 will be approximately 2 hours

Study Visit 2

On the second visit, the participant will report to the CTSI CRU. Baseline vital signs (BP, HR, Resp, POX) will be obtained for a baseline measurement, a catheter inserted into a peripheral vein in the upper extremity and pain report to 5 min heating of skin on the calf at the temperature determined in Visit 1 will be recorded. Subjects will then receive two 10 min infusion of study drug, with infusions separated by 60 min and 5 min heat pain testing performed with each 5 min test ending at 15, 30, and 45 min after the end of each infusion. Subjects will be randomized to receive either **1)** IV infusion of saline followed by oxytocin, 1.3 µg or **2)** IV infusion of oxytocin 0.3 µg followed by oxytocin 7 µg. Which of these regimens is administered on study visit 2 will be randomized and double blind, with study drugs provided by the research pharmacy. The intravenous catheter will be removed after all data collection and the subject discharged from the CRU. The duration for study visit 2 will be approximately 4 hours.

Study Visit 3

On the third visit, the participant will report to the CRU and all procedures performed as in study visit 2. The pair of IV infusions will be the alternate to that used in study visit 2. The duration for study visit 3 will be approximately 4 hours.

Safety and Monitoring:

Assessment of Side Effects*:* Significant side effects are defined as changes > 30% from baseline in the mean arterial pressure, HR, or oxyhemoglobin pulse oximetry < 90. Any unexpected or serious side effects will be reported to the IRB within 24 hrs. Peripheral oxyhemoglobin saturation, BP and HR will be measured non-invasively before and 15, 30, and 60 min after each IV infusion. If either BP or HR change by > 30%, or oxyhemoglobin saturation decreases to less than 90, and require treatment, these vital signs will be repeated at 5-minute intervals until vital signs are stabilized.

Treatment of Side Effects: Mean blood pressure elevations greater than 30% of baseline will be treated with labetalol 5-15 mg IV incrementally until BP elevation is less than 30% elevated. Reductions in mean BP > 30% of baseline will be treated with incremental ephedrine 5-20 mg, IV. Heart rate (HR) reductions or elevations > 30% of baseline will be treated with incremental glycopyrrolate 0.2 mg IV or propranolol 0.2 mg IV, respectively. Peripheral oxyhemoglobin

desaturation (< 90) will be treated with supplemental oxygen, nasal cannula, face mask, or non-rebreathing mask depending upon the degree desaturation and the response to therapy.

Outcome Measure(s)

Primary Outcome Measure

Name: Verbal pain score (0-10) at the end of 5 min of skin heating

Type: Primary

Time Frame: For 45 minutes after the end of each study drug infusion

Description: Pain scores are measured each minute during 5 min heating of the skin of the calf

Analytical Plan

The primary analysis will use NONMEM to create a dose response at the time of peak oxytocin effect on pain at the end of a 5 min noxious heat stimulus. To do so, pain scores over time will first be modeled using NONMEM with derived parameters of maximum effect (Emax), predicted concentrations from the PK/PD model to produce a 50% and 90% maximum drug effect (C50 and C90, respectively), the steepness of the dose response curve (γ), and the time to peak effect. Inter-subject variability (e.g., biological variability) will evaluate additive, proportional, and exponential models. Residual intrasubject variability (e.g., noise) will typically require an additive and multiplicative error model. The influence of covariates (e.g., age, sex, weight) on model parameters will be analyzed by serial inclusion / exclusion, with significance determined by the likelihood ratio test ($p < 0.01$ if the decrease in -2 log likelihood exceeds 6.6 ($\chi^2_{0.99}, \mathrm{df} = 1$)). Parameters will be estimated using first-order conditional estimate with η-ε interaction.

Covariate effects deemed significant by likelihood ratio test will be validated using jackknife cross-validation [10]. Jackknife cross-validation provides an estimate of the utility of the covariate to inform the pharmacodynamics in a future individual, rather than merely characterize the pharmacodynamics in the individuals from which the model was derived. The jackknife cross-validation divides the data into N subsets. Each subset leaves out a single individual. The parameters of the model are re-estimated for each subset with and without the covariate being validated. The inaccuracy (mean ((|measured – predicted|) / predicted)), of the model with and without the covariate is measured in the excluded individual. The covariate is only being included in the final model if it the improvement in model accuracy in the N iterations is significantly greater than 0. Jackknife cross-validation helps compensate for NONMEM's ability to identify very small covariate effects that do not meaningfully contribute to accuracy in future patients.

Expected outcomes. Based on pain ratings and an estimate of the PK/PD model from preliminary data (Fig 1), we anticipate that the maximum oxytocin effect is a 50-60% reduction in pain rating from the 5 min heat stimulus. We anticipate that the oxytocin dose range will demonstrate a sigmoidal dose-response with a peak within 10 min after the end of infusion and an ED80 of ~5 µg. In a separate simulation of 7 µg oxytocin without a prior injection of 0.3 µg, the peak effect was within 1% of the same value, supporting this stepped dosing study design. The expected peak plasma oxytocin concentration after the 7 µg infusion following the 0.3 µg infusion is 400 pg/ml, below that which produced subjective symptoms in previous studies.

Human Subjects Protection

Subject Recruitment Methods

Healthy subjects will be recruited from our current database of volunteers BG05-468, word of mouth and Be Involved website; The research nurses; Regina Curry, RN and Vonda McGee, LPN will conduct the recruitment of study subjects.

Potential subjects from the database will be contacted via phone or email, per their request. Potential subjects from the Be Involved website will be contacted according to the information they provide.

Informed Consent

Signed informed consent will be obtained from each subject. Regina Curry, RN or Vonda McGee, LPN will obtain consent. Potential subjects will be consented in the CRU. A private room will be utilized during the consent process and all study visits.

Confidentiality and Privacy

Confidentiality will be protected by collecting only information needed to assess study outcomes, minimizing to the fullest extent possible the collection of any information that could directly identify subjects, and maintaining all study information in a secure manner. To help ensure subject privacy and confidentiality, only a unique study identifier will appear on the data collection form. Any collected patient identifying information corresponding to the unique study identifier will be maintained on a linkage file, store separately from the data. The linkage file will be kept secure, with access limited to designated study personnel. Following data collection subject identifying information will be destroyed 3 years after closure of the study, in confidential shredding disposal bins consistent with data validation and study design, producing an anonymous analytical data set. Data access will be limited to study staff. Data and records will be kept locked and secured, with any computer data password protected. No reference to any individual participant will appear in reports, presentations, or publications that may arise from the study.

Data and Safety Monitoring

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. The principal investigator will be assisted by other members of the study staff.

The DSMP outlined below will provide appropriate oversight and monitoring to ensure the safety of participants, the validity of the data, and make intermittent recommendations whether to continue, modify or stop the study. The DSMP will utilize an independent DSMB to ensure the effective institution of the DSMP.

This DSMB will have discretion to unblind any results, or conduct any inquiry needed to ensure the safety and efficacy of the trial at the request of the DSMB chair. The committee will maintain a written record of its meetings.

Scope of Data Monitoring

The primary source of the data will be adverse event reporting.

Study admission data
Monitoring of admission data will include the number of subjects requesting participation in the study, number of subjects screened and number of subjects admitted to the study. The DSMB may request a report of the reason why subjects were disqualified from participating in the study. For subjects admitted to the study, the DSMB will review eligibility criteria for admitted subject, any protocol deviations and/or violations, and the demographic distribution of the subjects by group.

Protocol Compliance
The DSMB will monitor the data to assess compliance with the protocols including the adherence to the randomization schedule. The DSMB will also monitor the quality and completeness of the data being collected, including the frequency of missing or erroneous data, and presence and frequency of outliers.

Safety Data
Monitoring of safety data will include review of Adverse Events (AEs) and Serious Adverse Events (SAEs), trial retention, and reason for drop out. Safety information will be reported to the DSMB in an unblinded manner. Formal statistical analyses of the safety data may be requested by the DSMB. For SAEs, data will include all the adverse event data meeting the FDA definition of serious adverse events. In the assessment of SAEs, the DSMB will review each individual case including treatment group assignment. After each meeting of the DSMB, the secretary will forward a summary report of all serious and unexpected adverse experiences to the principal investigator to summarize the DSMB's review of the serious and unexpected adverse events reported. Furthermore, the DSMB will make a recommendation to continue, modify or halt the study protocol. This report will be transmitted to the Wake Forest University IRB and NIH. Safety data will be prepared for review following the enrollment of each 24 subjects.

Establishing a DSMB Board membership
The DSMB will be appointed by Dr. Eisenach and Dr. Ririe with the purpose of reviewing, approval, and monitoring the implementation of the DSMP. The DSMB will have two members encompassing multidisciplinary expertise who are not involved in the study protocol. Board members will have no financial and/or scientific ties to the outcome of the clinical trials to avoid any real or perceived conflict of interest. The DSMB will include the following physicians; Laura Dean, M.D., Associate Professor of Anesthesiology and Director of Obstetric and Gynecologic Anesthesiology and Ashish Khanna, M.D., Associate Professor and Vice Chair for Research in Anesthesiology, Wake Forest School of Medicine.

Board meeting schedule
The board will have scheduled meetings twice a year and expedited meetings to review unexpected SAEs or other urgent issues that may arise during the trial. Unscheduled meetings may be initiated by the DSMB chair, Dr. Eisenach, or Dr. Ririe. The data to be reviewed by the DSMB will be available to the Board members.

DSMB Recommendations:
DSMB recommendations will be made in writing by The DSMB chair to Dr. Eisenach. The secretary will prepare meeting minutes for inclusion in the DSMB report. The draft report will be reviewed by all Board members prior to issuance of the final report. DSMB recommendations will then be forwarded to the NINDS program officer and Wake Forest University IRB.

Volunteer Payment

Participants will be paid a total of $400 according to the following payment schedule which we have used throughout the last 10 years. We believe that this payment schedule is fair and appropriate, paying for each procedure attempted and an additional payment for completion of the entire study.

Completion of study visit 1: $50
Study visit 2: placement of IV-$25, IV infusion and thermal testing-$100
Study visit 3: placement of IV-$25, IV infusion and thermal testing-$100
Completion of all study related procedures: $100

If an IV cannot be placed on Study visit 2: subject will be compensated $50 and subject replaced

Long-term Follow-up

Volunteers will be contacted daily within the following week and questioned about adverse events from the study.

Reporting of Unanticipated Problems, Adverse Events or Deviations

Any unanticipated problems, serious and unexpected adverse events, deviations or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB, DSMC and the FDA if appropriate.

References

1. Eisenach, J.C., et al., *Resolution of pain after childbirth.* Anesthesiology, 2013. **118**(1): p. 143-51.
2. Aschenbrenner, C.A., et al., *Modeling individual recovery after peripheral nerve injury in rats and the effects of parturition.* Anesthesiology, 2014. **121**(5): p. 1056-67.
3. Gutierrez, S., et al., *Reversal of peripheral nerve injury-induced hypersensitivity in the postpartum period: role of spinal oxytocin.* Anesthesiology, 2013. **118**(1): p. 152-9.
4. Boada, M.D., et al., *Nerve injury induces a new profile of tactile and mechanical nociceptor input from undamaged peripheral afferents.* J Neurophysiol, 2015. **113**(1): p. 100-9.
5. Boada, M.D., S. Gutierrez, and J.C. Eisenach, *Peripheral oxytocin restores light touch and nociceptor sensory afferents towards normal after nerve injury.* Pain, 2019. **160**(5): p. 1146-1155.
6. MacLean, E.L., et al., *Challenges for measuring oxytocin: The blind men and the elephant?* Psychoneuroendocrinology, 2019. **107**: p. 225-231.

7. Fernando, D., et al., *Safety, Tolerability and Pharmacokinetics of Single Doses of Oxytocin Administered via an Inhaled Route in Healthy Females: Randomized, Single-blind, Phase 1 Study.* EBioMedicine, 2017. **22**: p. 249-255.
8. Martins, D.A., et al., *Effects of route of administration on oxytocin-induced changes in regional cerebral blood flow in humans.* Nat Commun, 2020. **11**(1): p. 1160.
9. Boada, M.D., D.G. Ririe, and J.C. Eisenach, *Post-discharge hyperpolarization is an endogenous modulatory factor limiting input from fast-conducting nociceptors (AHTMRs).* Mol Pain, 2017. **13**: p. 1744806917726255.
10. Fiset, P., et al., *Pharmacokinetics of computer-controlled alfentanil administration in children undergoing cardiac surgery.* Anesthesiology, 1995. **83**(5): p. 944-55.