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A Study of Oxytocin Pharmacokinetics after Intravenous and Intranasal Administration in Healthy Subjects

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Background, Rationale and Context

Oxytocin (OXT) uniformly produces antinociception and anti-hyperalgesia in rodents, yet intranasal OXT fails to reduce pain in 6 of 13 clinical studies [2]. Three observations from our group suggest that OXT is not analgesic in the normal condition, but speeds recovery from pain after injury and prevents the transition from acute to chronic pain: **1)** The incidence of chronic pain from surgery is considerably lower after cesarean section compared to other pelvic surgeries [3], **2)** recovery from hypersensitivity after nerve injury is quicker in female rats when injury occurs at the time of delivery, an effect temporarily reversed with a nonselective OXT and vasopressin 1a (V1a) receptor antagonist [4], and **3)** selective antagonists of these receptors temporarily reverse recovery from nerve injury in both male and female rodents [5].

OXT itself is the sole readily-available tool to test clinical relevance of basic science studies of OXTergic signaling in other neurologic disorders, including pain, neuroprotection during brain ischemia, ADHD and autism and in psychiatry, including social behaviors which are positive (trust, neonatal-maternal and adult pair bonding) and negative (aggression and ethnocentrism). Existing clinical studies rely primarily on correlations between disease activity and common polymorphisms in OXT receptors or plasma OXT concentrations, which are unlikely to reflect OXT release in the brain.

Direct evaluation of peripheral and central effects of systemically administered OXT in humans is hampered by bolus dosing, lack of dose response, and ignorance of peripheral and central drug distribution, especially with intranasal administration. Poor rigor and fundamental ignorance of drug disposition partly underlie the uncertainty regarding OXT's actions in acute and chronic pain.

Prior to 2016, and to a considerable extent since, OXT was and is measured with antibody-based methods (EIA, ELISA, or RIA). Reported concentrations vary widely from study to study and, since development of accurate LC/MS assays, there is now considerable skepticism regarding the validity of the previous research and concerns that the older methods measure OXT degradation fragments (see [6] for review). Note in **Figure 1** the high baseline and wide variability in OXT concentrations and markedly different change in OXT concentrations after intranasal (i.n.) administration when ELISA and RIA methods are used compared to that of LC/MS, which unamiguously measures OXT [4; 5; 8; 9].

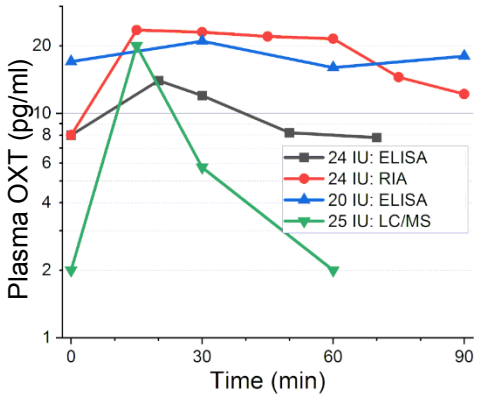

Figure. 1. Mean plasma OXT via i.n. administration by various assays.

In preliminary data we infused OXT, 17 µg, iv in 11 healthy men and women aged 22-58 years, measured plasma OXT with both LC/MS and with ELISA using validated protocols, and modeled the resultant data using NONMEM. Transient headache and facial flushing occurred when this dose was injected over 1 min in the first 3 individuals and these effects were reduced or eliminated with infusion over 10 min in the remainder of the subjects. PK analysis showed no effect of sex and a minor effect of body weight. Modeled data from the LC/MS analysis differ significantly from those using RIA assays after iv OXT injection [2; 7], which themselves differ widely (**Figure 2A**). In our study, ELISA assays showed more inter-individual variability (NONMEM estimates of variance due to assay for ELISA was 28% compared to 16% for LC/MS) and considerably different PK parameters than those obtained from LC/MS assays. Simulated target controlled infusions (TCI) based on

these models demonstrate the greater variability with ELISA than LC/MS (**Figure 2B**). At the same total dose of 17 µg OXT, a 30 min TCI infusion using PK parameters using LC/MS assays would result in a targeted concentration nearly double that using PK from ELISA assays (80 vs 46 pg/ml for LC/MS vs ELISA).

Defining PK of OXT in plasma after peripheral administration is required in order to determine PK / pharmacodynamic (PK/PD) relationships for actions centrally, the primary focus for much of neuroscience OXT research, and peripherally, the focus of our primary hypothesis regarding analgesic and disease-modifying effects of OXT in pain after injury. This requires measuring OXT with a reliable, sensitive, and specific assay to define PK and using the knowledge of PK to design dosing schemes with reliable measures of drug action at sites of interest. It also requires creation of outcome measures in the periphery (beyond those peculiar to obstetrics) and centrally which can be repeatedly measured at frequent intervals adequate for PK/PD modeling. These are the goals of this clinical project within a multi-project NIH proposal (P01-NS119159).

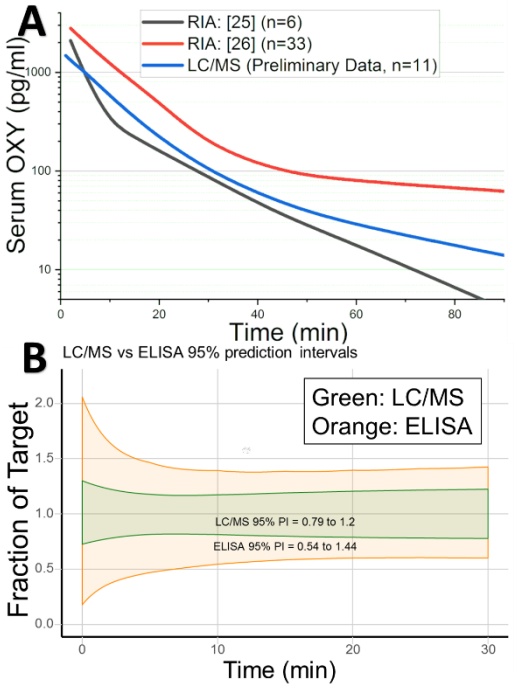

Figure 2. A) PK modeled OXT concentrations in preliminary data compared with published reports. **B)** Fraction of targeted concentration using TCI. 95% prediction intervals from LC/MS or ELISA assays of duplicate samples (n=11).

I.n. administration is far more practical for subacute and chronic treatment, as would be needed in clinical trials, and the purpose of the current study is to determine the bioavailability and PK of OXT after i.n. administration. The proposed study utilizes a multi-visit, crossover design. In order to determine bioavailability, each subject will receive OXT by intravenous injection on one occasion and i.n. on others. To determine linearity of OXT bioavailability across doses to be subsequently studied, two i.n. OXT doses will be administered on different occasions. Subjects will be stratified across three age groups from young adults to the elderly, with balanced recruitment by sex in order to assess covariates of age and sex in OXT PK.

Objectives

Main objective Model OXT concentrations in plasma after intravenous and i.n. administration in order to determine absorption rate and bioavailability of OXT in plasma after i.n. administration.

Additional Objectives

1. Describe adverse events with intravenous and i.n. administration

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).

Subjects will report to the CTSI Clinical Research Unit (CRU) for two visits, separated by at least 5 days. On study visit one informed consent will be obtained and eligibility verified. Then two intravenous (IV) catheters will be inserted, one in each forearm, with one for drug infusion and the other for blood sampling. After baseline measures, subjects will receive oxytocin (Pitocin®), 14 µg (8.2 IU) over 30 minutes. This dose and infusion duration were chosen based on previous studies showing no adverse events in 10 volunteers and our preliminary PK model which predicts that plasma oxytocin concentrations will be above the limit of detection (1 pg/ml) at 120 min. Venous blood samples (5 ml) will be obtained at 2, 5, 10, 20, 30, 40, 50, 65, 90, and 120 minutes after the initiation of oxytocin administration for oxytocin assay.

On study visit 2, one IV catheter will be placed for blood sampling. After baseline measures, subjects will receive 102 µg (60 IU) intranasal oxytocin (Tonix-1900, Tonix Pharmaceuticals, Chatham, NJ). Venous blood samples (5 ml) will be obtained at 1, 2, 5, 7, 10, 15, 20, 25, 35, 45, and 60 minutes after the initiation of the oxytocin administration for oxytocin assay. See graph 3 for rationale for the times of sampling.

We will abandon an individual participant's study and schedule another participant should we be unable to successfully place the intravenous catheters.

STUDY DESIGN

This is a sequential study of subjects, all of whom will receive, on different occasions, an intravenous infusion or intranasal (i.n.) sprays of oxytocin. Blood samples will be obtained thereafter in order to create a mathematical model to describe the concentrations of oxytocin in the blood over time (pharmacokinetics). In this study, healthy volunteers are recruited for two study drug visits. They will come to the CRU and have two IV catheters inserted, one in each arm for the first study visit. They will receive a 30 minute infusion through one of the IV catheters of oxytocin, 14 µg, and blood will be obtained at several times over the next 120 minutes and the amount of oxytocin measured in the blood samples. They will come a second time to the CRU and have one IV inserted in their arm and self-administer i.n. oxytocin, 102 ug over 5 min and blood will be obtained several times over the next 60 minutes and the amount of oxytocin measured in the blood samples. Plasma oxytocin concentrations and individual subject characteristics (age, sex, weight, height, race, and ethnicity) will be analyzed by another group at Stanford University funded under the same NIH grant supporting the work at Wake Forest. The effect of these subject characteristics on the pharmacokinetics of oxytocin will be examined, since these characteristics alter concentration over time of some drugs. Should these characteristics alter oxytocin concentrations, we and others could adjust the dose of oxytocin to the individual.

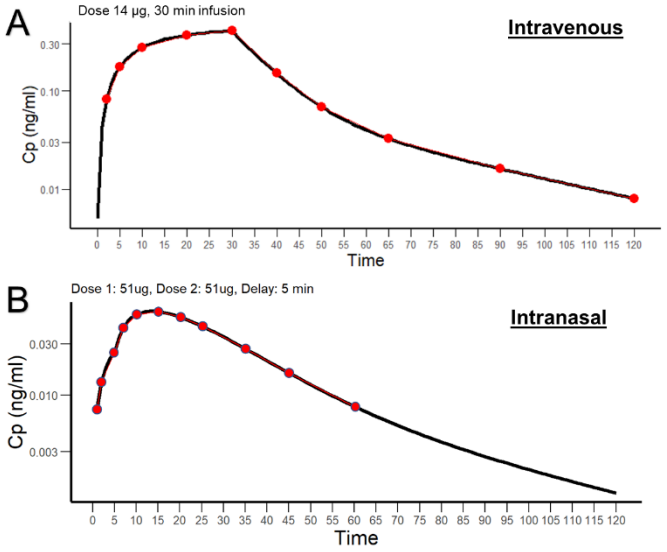

Figure 3. Simulated PK and blood sampling times
A) Simulated plasma OXT concentrations (Cp) with IV infusion. The proposed sampling times (red circles) optimize the informational content to confirm the model generated from 11 subjects in an earlier study.
B) Simulated plasma OXT concentrations (Cp) with I.N. self-administration. The proposed sampling times (red circles) optimize the informational content to capture the absorption rate constant. The simulated model assumed a Cmax of 15 min and 1% bioavailability.

The main purpose of this study is to sample blood and calculate the pharmacokinetics of oxytocin. We will also assess adverse events during the study.

The research participants will not benefit from this study, but the knowledge we get will be important not only to adjust oxytocin dose to individuals, but to study its possible effects on pain in a more precise way by controlling the amount of oxytocin in the blood circulation. The sample size we chose is needed to get an accurate estimate for the parameters in the pharmacokinetic model for the population in general, not just the subjects in this study.

Setting

Study visits will occur on the main campus at Atrium Wake Forest Baptist Health in the Clinical Research Unit (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 or 2.
3. For healthy volunteers, normal blood pressure (systolic 100-140 mmHg; diastolic 60-90 mmHg) resting heart rate 45-90 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.

4. 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 urine pregnancy test at visit 1), women who are currently nursing or lactating, women that have been pregnant within 2 years
4. Subjects with neuropathy, chronic pain (being treated on a daily basis), 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.
8. History of chronic nasal obstruction or local pathology in nostril pathway which, in the opinion of the investigator, would prevent appropriate nasal administration of the study drug.
9. Use of OTC nasal products (ie. Saline spray, Neti-Pot, etc.) or intranasal corticosteroid medications during the study.

Sample Size

We request permission to study up to 30 subjects so that we will have 24 evaluable subjects, respectively, with complete sampling at all time periods. Sample size will be divided into 3 groups; young (18-39-year-old) middle (40 – 59-year-old) and older (60 – 75-year-old).

N	Parameter	Mean	95% Confidence Bound		Range	Correction	Corrected Range
			Lower	Upper			
11	Cl1	0.98	0.89	1.08	20%	2.54	50%
	V1	10.2	8.9	11.9	29%		74%
	Cl2	0.21	0.18	0.24	29%		74%
	V2	7.1	6.1	8.1	28%		70%
20	Cl1	0.97	0.95	1.01	7%	1.88	12%
	V1	10.1	9.6	10.6	10%		18%
	Cl2	0.20	0.20	0.21	8%		16%
	V2	7.0	6.7	7.3	8%		16%

Table 1 Bootstrap parameter estimates of PK model.

Sample size was determined using of bootstrap analyses and conventional calculations to estimate the pharmacokinetic parameters within the desired level of accuracy. Table 1 shows the results of 1000 bootstrap replications of the original 11 individuals in the pharmacokinetic study, resampled with N = 11 (the original study size) and N = 20. As expected, the estimates are more precise with increasing study size. The mean and 95% confidence intervals are shown for each parameter.

The 95% confidence intervals from the bootstrap are corrected to provide a "best" estimate of the true 95% CI using the upper confidence limit (UCL) method of Browne [1]. As described by Whitehead [10], the 95% UCL correction is k/χ_(0.95,k)^2 where k is N-1, the number of degrees of freedom. As seen in Table 1, the parameters estimated for the initial 11 subjects have a reasonable level of certainty (95% CI < 30% of the mean value) for a small study. However, the corrected CI range from the bootstrap for an N=11 study is closer to 70%. A study with 20 subjects will estimate the parameters within a corrected 95% CI range within 20% of the mean estimated value (Table 1, numbers in red). This is sufficient accuracy to inform the subsequent studies.

Sample size in this study is increased to 24 permit stratifying by age (3 strata), weight (2 strata), and sex. A corrected confidence range less 20% (Table 1) implies that mixed effect analysis will distinguish covariate effects in the range of 40% in a study with 20 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.

Interventions and Interactions

Study Visit 1: The participant will report to the Clinical Research Unit (CRU) of the Wake Forest Clinical Translational Science Institute (CTSI), in the morning after having had nothing to eat or drink since midnight. 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 peripheral oxygen saturation (POX) will be measured and recorded along with height and weight.

Once eligibility has been determined; baseline vital signs (BP, HR, Resp, POX) will be obtained for a baseline measurement and then at the following intervals after the infusion has been initiated; 5, 10, 30, 60, 90 and 120 minutes. Two intravenous catheters will be inserted, one in each arm, and baseline measurements will be obtained. Then 14 µg (8.2 IU) oxytocin (Pitocin®), obtained from the research pharmacy, will be infused over 30 min through one of the catheters. Blood (5 ml) will be sampled from the other catheter at 2, 5, 10, 20, 30, 40, 50, 65, 90, and 120 minutes after the initiation of oxytocin, placed on ice, plasma separated in a refrigerated centrifuge, and stored at -80°C until analyzed for oxytocin concentration. The study will not be blinded. The intravenous catheters will be removed after all data collection and the subject discharged from the CRU. The duration for study visit 2 will be approximately 3.5 hours.

Study Visit 2

On the second visit, the participant will report to the CRU, in the morning after having had nothing to eat or drink since midnight. Baseline vital signs (BP, HR, Resp, POX) will be obtained for a baseline measurement and then at the following intervals after the intranasal oxytocin: 10, 20, 30, 60, 90 and 120 minutes. One intravenous catheter will be inserted. Oxytocin, 102 µg (Tonix-1900, Tonix Pharmaceuticals, Chatham, NJ), obtained from the research pharmacy, will be self-administered in the nasal passages as one metered spray in each nostril followed in 5 min by a second metered spray in each nostril. Blood (5 ml) will be sampled from the IV catheter at 1, 2, 5, 7, 10, 15, 20, 25, 35, 45, and 60 minutes after the initiation of the oxytocin administration, placed on ice, plasma separated in a refrigerated centrifuge, and stored at -80°C until analyzed for oxytocin concentration. 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 3 hours.

Safety and Monitoring:

Assessment of Side Effects*:* Peripheral oxyhemoglobin saturation, BP and HR will be measured non-invasively before and 5, 10, , 30, 60, 90 and 120 minutes after oxytocin injection and before and 10, 20, 30, 60, 90 and 120 minutes after the intranasal administration. Subjects will informed to report any subjective sensations during or after oxytocin administration and will be queried at the same intervals as vital sign monitoring for any subjective sensations. At the end of Study Visit 2 the nasal passages will be inspected for any localized tissue reaction.

Treatment of Side Effects: Significant cardiorespiratory side effects include:

a. Decreased BP with symptoms of hypotension (e.g., dizziness or lightheadedness)
b. Blood pressure or HR changes >20%
c. Pulse oximetry less than 94% that does not correct with several deep breaths.

Treatment of BP elevations > 20% of baseline will be treated with labetalol 5-15 mg IV incrementally until BP elevation <20% elevated. Treatment of symptomatic hypotension or BP reductions >20% will be treated with

incremental ephedrine 5-20 mg, IV. Heart rate (HR) reductions or elevations > 20% 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.

Regardless of whether any treatment is required, subjects with significant side effects will be monitored more frequently (every 5 min for BP and HR; continuously for POX) until symptoms or signs resolve.

Serious adverse events: In addition to treatment, as described above, and timely reporting as required to the DSMC, IRB, and FDA, emergency treatment will be available within the CRU itself. As such, an ACLS-certified health care professional will be on site or within 5 minutes of the CRU during each treatment session, and a crash cart containing medicine and equipment for emergency resuscitation, and an automated external defibrillator will be located on-site.

Study Stopping Criteria: Should significant cardiorespiratory or other side effects occur during oxytocin administration, the IV infusion or intranasal self-administration will be stopped and the subject treated as indicated in Treatment of Side Effects. Blood samples will be obtained near the time of oxytocin cessation and according to the sampling intervals of the study once the side effects are successfully treated or resolve.

The study will be stopped if more than 2 individuals exhibit significant cardiorespiratory side effects or after any serious adverse event and future conduct of the study will be determined by recommendations from the DSMC, IRB, and FDA.

Outcome Measure(s)

Primary Outcome Measure
Name: Oxytocin concentration in plasma
Type: Primary
Time Frame: For 120 minutes after the end of the beginning of intravenous oxytocin infusion
Time Frame: For 60 minutes after the administration of the 102 µg intranasal oxytocin

Description: Blood will be sampled at specified intervals during and after the IV infusion and after the intranasal self-administration. Plasma will be separated, rapidly frozen, and later analyzed for oxytocin concentration. The oxytocin concentrations will be modeled to describe the pharmacokinetics of oxytocin and the bioavailability of oxytocin after i.n. administration. We will also determine the effect, if any, of the covariates sex, age, weight, race, and ethnicity.

Analytical Plan

The parameters of the PK model will be fit to the observed plasma concentrations using NONMEM. 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 ($\chi 0.99\ 2$, 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 crossvalidation [3]. Jackknife cross-validation provides an estimate of the utility of the covariate to inform the pharmacokinetics in a future individual, rather than merely characterize the pharmacokinetics 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 included in the final model if it the improvement in model accuracy in the N iterations is significantly greater than 0. Jackknife crossvalidation helps compensate for NONMEM's ability to identify very small covariate effects that do not

meaningfully contribute to accuracy in future patients. We will study 24 subjects. Bootstrap analysis indicates that the confidence interval for studies of 20 individuals using the LC/MS assay will determine the PK parameters with a corrected confidence range < 20%. A corrected confidence range less 20% implies that mixed effect analysis will distinguish covariate effects in the range of 40% in a study with 20 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 i.n. bioavailability and dose linearity despite expected high inter-subject variability in absorption.

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 Sharing Plan

Plan Description: Statistical programs in the data analysis will be written in R. The software and anonymized data will be included both as digital supplements in the published papers and will be posted on GitHub under github.com/StevenLShafer. Interested investigators will be able to reproduce the published analyses from these files. Consistent with the posting of software and data to the OpenTCI initiative, the software and de-identified data will be made available with "no strings attached," enabling investigators to freely use the data to inform or supplement additional research without restriction. Data to be made available are oxytocin dose, times of sampling (relative to the start of dosing), plasma oxytocin concentrations at those times for each subject, and age, weight, and sex for each subject.

Data and Safety Monitoring

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. A Data Safety Monitoring Committee will include Laura Dean, M.D., Associate Professor, Section Head Obstetric and Gynecologic Anesthesia and Ashish Khanna, M.D., Associate Professor, Anesthesiology. A cumulative report will be sent to the committee to review quarterly and all serious and unexpected adverse events will be reported to the committee for review and recommendations.

Volunteer Payment

Participants will be paid a total of $500 according to the following payment schedule which we have used throughout the last 2 cycles of this grant's protocols. 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.

Study Visit 1: 2 PIV $25/each ($50); $100 for PK sampling
Study Visit 2: 1 PIV $25; $100 for PK sampling + $225 for completion of study visit 2 PK sampling

Subject must complete all of the above phases to receive this payment.

If 2 PIV's cannot be placed on Visit 1: subject will be compensated $50 and subject replaced

Long-term Follow-up

Volunteers will be contacted daily for one 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 FDA as required by each.

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