BACKGROUND

There is a strong experimental basis to support the study of oxytocin by the spinal route for analgesia in humans. Oxytocin containing cells in the dorsal parvocellular division of the paraventricular nucleus (PVN) project to the spinal cord (1). Noxious stimulation activates these cells via the A1 noradrenergic relay in the pons (2) and produces analgesia by spinal release of oxytocin, since intrathecal injection of an oxytocin receptor antagonist worsens pain behaviors from peripheral inflammation (3). Direct electrical stimulation of the PVN reduces dorsal horn neuronal responses to noxious stimulation, and this is blocked by administration of sequestering antibody for oxytocin (4). Similarly, direct electrical stimulation of the PVN reduces behavioral sensitivity in a model of chronic neuropathic pain, and this effect is blocked by an oxytocin receptor antagonist (5). Intrathecal injection of oxytocin in normal rats reduces dorsal horn neuronal responses to noxious stimuli (6) as well as behavioral responses to noxious thermal (3), mechanical (3), and chemical (7) stimuli. Finally, intrathecal injection of oxytocin in rat models of chronic pain also reduces dorsal horn neuronal responses to sensory stimulation (6) as well as behavioral responses to thermal (5) and mechanical (7) stimuli.

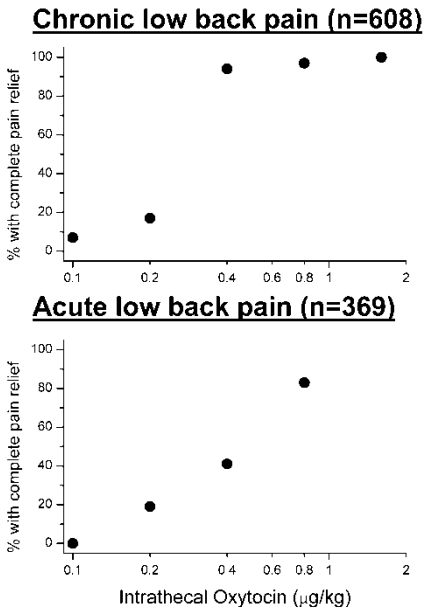

Figure 1. Percentage of patients with complete pain relief from intrathecal injection of oxytocin.

Many compounds and manipulations produce evidence of analgesia in rodents, but most studies, including the ones cited for oxytocin above, rely on reflex withdrawal responses to simulation or electrophysiologic responses in individual cells. As such, the relevance of these observations to spontaneous pain in humans is far from certain. Two observations, however, strongly suggest that intrathecal oxytocin would be analgesic in humans.

The first, most direct evidence, is a report from China of 608 patients with chronic low back pain and 369 patients with acute low back pain who received single intrathecal injections of oxytocin (8). Intrathecal oxytocin produced acute analgesia in both settings, with nearly

universal complete pain relief reported at doses of approximately 30 µg per injection for chronic low back pain and 60 µg per injection for acute low back pain (Figure 1). Duration of analgesia was dose dependent, with < 1 hr analgesia from approximately 7 µg per injection and > 5 hr analgesia from approximately 60 µg per injection.

The second line of evidence comes from our clinical observations in obstetric patients. Physical injury, whether from trauma or surgery, is recognized as an important cause of chronic pain, and the incidence of chronic pain after surgery ranges from 10 to 40%, depending on the procedure (9). We recently completed a clinical trial of over 1200 women examining the incidence of chronic pain after vaginal and cesarean delivery. Although approximately 10% of women had pain 2 months after delivery (10), the incidence of pain one year after delivery was remarkably low (95% confidence limit for pain at one year of 0.7%). This is at least an order of magnitude lower than that reported from any other abdominal surgery, including superficial surgery like inguinal herniorrhaphy (9). We hypothesized, based on these studies, that a factor which is present during pregnancy may be protective of chronic pain. We therefore examined hypersensitivity in rats following a surgery (spinal nerve ligation) which is commonly used as a model of neuropathic pain. To our surprise, this surgery produced similar degrees of hypersensitivity when performed during pregnancy than in nonpregnant animals (Figure 2). In contrast to this lack of effect of pregnancy, the degree of hypersensitivity significantly diminished for several weeks after delivery, abruptly returning when the pups were weaned from

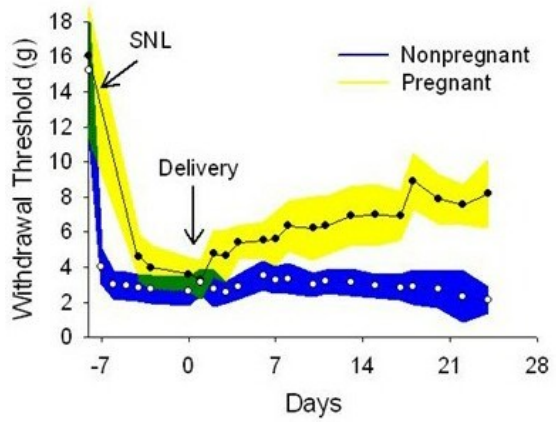

Figure 2. Withdrawal threshold drops similarly after spinal nerve ligation (SNL, a model of neuropathic pain) in pregnant and nonpregnant animals, but this hypersensitivity is alleviated after delivery. Colored areas are 95% CIs.

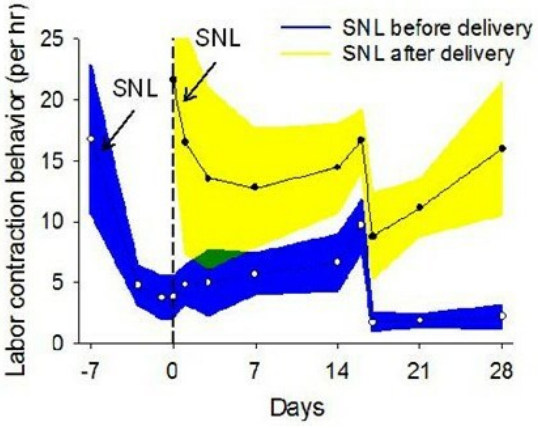

Figure 3. In animals with SNL performed during pregnancy, withdrawal threshold drops abruptly when pups are weaned at 17 days, but when SNL is done on the day of delivery, withdrawal threshold never changes from control.

the dams (Figure 3). When nerve injury surgery was performed on the same day as delivery, hypersensitivity never developed, mimicking the clinical trial results of a lack of chronic pain from surgical or vaginal delivery (Figure 3).

We subsequently performed several studies which suggest that spinal release of oxytocin represents the protective factor against chronic pain after childbirth. Intrathecal injection of the specific oxytocin receptor antagonist, atosiban, temporarily reverses the blockade of nerve injury induced hypersensitivity in the postpartum period in rats. Similarly, intracerebroventricular administration of the α-adrenoceptor antagonist, phentolamine, which is known to block the effects of lactation on activation of oxytocin-containing neurons in the paraventricular nucleus (PVN) of the hypothalamus, also temporarily reverses the blockade of nerve injury induced hypersensitivity in the postpartum period in rats. Finally, protection against hypersensitivity from nerve injury is abruptly reversed when pups are separated from dams for 24 hr, and this is accompanied by a large decrease in oxytocin concentration in cerebrospinal fluid. Although we are currently performing more clinical and laboratory studies to further define this mechanism, these data suggest that spinal oxytocin receptor stimulation in the period immediately following delivery protects against the generation of injury-induced hypersensitivity and subsequent chronic pain.

We recently completed a Phase 1 safety study of intrathecal oxytocin and observed no serious adverse events over the dose range studied (5-150 μg). One subject at the 150 μg dose had transient subjective numbness in sacral dermatomes, but this was preceded by a vasovagal episode beginning just prior to spinal injection, and this did not occur in other subjects at this dose. There were no objective signs of motor or sensory dysfunction in any volunteer, and blood pressure, heart rate, sensorium, corrected QT interval and serum sodium were unaffected.

We anticipate that oxytocin will be effective after spinal injection in humans against chemical induced hypersensitivity states. For these future studies, we will use a controlled and blinded study of intrathecal oxytocin against topical capsaicin, which we have used in previous studies under IND of spinal analgesics and which results in an area of increased pain sensation to a normally painful stimulus (hyperalgesia) and pain sensation to what previously was an innocuous stimulus (allodynia). This model of superficial and deep neuropathic pain in humans can be easily studied in volunteers, as these sensations last only a few hours and are not

distressing. Spinal oxytocin is effective in reducing or blocking experimentally induced hyperalgesia in rats, and we anticipate it will be in humans as well.

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Protocol

Efficacy of Intrathecal Oxytocin in Human Volunteers

GOAL: Determine the effect of intrathecal oxytocin on areas and intensity of hyperalgesia and allodynia induced by topical capsaicin.

METHODS: Equal numbers of healthy men and women will be recruited. At least 3 days prior to study subjects will be trained to rate pain in response to heat applied to the lateral calf using a Peltier controlled thermode, and to rate pain in response to 225 mN von Frey filament testing within the area of hypersensitivity induced and maintained by transient application of topical capsaicin followed by intermittent heat (Petersen, 1999).

Subjects will return for study visit two and be randomized into one of 3 groups of 12 subjects each to receive, in a double-blind manner, intrathecal saline or intrathecal oxytocin, 15 μg or 150 μg. The solutions will be prepared and dispensed to this institution under IND approval and regulation from the Food and Drug Administration and will be released to the investigators through the research pharmacy at Wake Forest School of Medicine.

A stimulus response to heat pain will be performed, then intrathecal injection of the study solution in a 3 ml volume will occur via a #27 or #25 Whitacre needle inserted in a lower lumbar interspace. Thirty minutes later capsaicin will be applied to the lateral calf to generate hypersensitivity. Capsaicin will be removed and the area re-kindled 40 min later by application of a thermode at 40ºC for 5 minutes. Areas of hyperalgesia and allodynia and, within the area of hyperalgesia, stimulus response to von Frey filament testing, will be determined upon removal of capsaicin and after 3 rekindlings at 40 minute intervals.

We will abandon an individual participant's study and schedule another participant to receive the same randomization, should we be unable to successfully place the intravenous catheter (IV) or the spinal needle to inject the intrathecal oxytocin. In our experience, this occurs in less than 10% of participants. We maintain an extensive listing (IRB approved Database BG 05-468: PI: James Eisenach, M.D.) of our previous Clinical Research Unit (CRU) volunteers who have requested to be notified by mail or email that they might qualify for participation in another volunteer study. It is from this list and potential volunteers that we maintain that we

anticipate recruitment of most of our volunteers. Should we need to advertise for additional volunteers we will submit a proposed advertising method to the IRB for approval.

STUDY DESIGN

Inclusion Criteria: We request permission to study up to 40 subjects so that we will have 36 evaluable subjects.

1. Male or female ≥ 18 and < 60 years of age, Body Mass Index (BMI) less than 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. Normal blood pressure (systolic 90-140 mmHg; diastolic 50-90 mmHg) resting heart rate 45-100 beats per minute) without medication.

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 the study drug, lidocaine or chili peppers.

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, or those who have delivered within 2 years.

4. Currently taking prescription or over the counter pain medications (opioid or non-opioid), diuretics, blood pressure medication, heart medication or any other medication at the discretion of the investigators that would place the subject at increased risk.

RANDOMIZATION GROUPS

A computer generated randomization will be used to determine the group for each subject. Subjects will be randomized to receive an intrathecal injection of the saline placebo or one of two doses oxytocin.

Study Visit 1: The participant will report to the Clinical Research Unit (CRU) at least 3 days prior to study visit 2. 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 blood drawn and sent to the laboratory to determine pregnancy status. The research nurse will train the participants to estimate pain quantitatively using a 2 cm^2 peltier controlled thermode (TSA®) applied to the forearm. Typically, the participant is exposed to temperatures between 38^0 and 51^0 C using a random staircase method.

The participant will also be trained to reproducibly define the limits of hyperalgesia and allodynia on the skin of the lateral calf established by heating the skin to 45ºC for 5 minutes, applying topical capsaicin (0.075% cream, Bioglan Pharmaceuticals) under an occlusive dressing for 30 minutes, then heating the application site to 40ºC for 5 minutes every 40 minutes (rekindling) as previously described (Petersen, 1999).

During this training period, the participant's blood pressure, heart rate, and peripheral oxyhemoglobin saturation will be recorded. A 12 Lead electrocardiogram (EKG) will also be performed.

We have found this training session significantly reduces anxiety and variability in pain and hypersensitivity ratings on subsequent study days.

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. A peripheral intravenous catheter will be inserted into a vein in an upper extremity and lactated Ringers solution infused at 1.5 ml/kg/hr for the duration of the study. After obtaining baseline measures the subject will be placed into a lateral position and one of the investigator physicians will insert a #25 or #27 Whitacre spinal needle in a lumbar interspace using a sterile technique. After obtaining clear, free-flow CSF, a 2 milliliter sample of CSF will be obtained and stored for future use under IRB Protocol: 00003383, the sample will be

stored with a unique identifier. Oxytocin in a 3 ml volume, diluted with sterile normal saline, or a 3 ml injection of sterile normal saline (placebo) will then be injected over 30 seconds. Oxytocin will be prepared by Med Solutions in Winston Salem, North Carolina and will be shipped to WFUHS for research study purposes only. The spinal needle will be removed and the volunteer placed supine with the head of the bed elevated for the next portion of the study. 30 minutes after the spinal injection the skin on the lateral calf will be heated to 45ºC for 5 minutes then topical capsaicin cream will be applied to the lateral calf to generate hypersensitivity and remain on the skin for 30 minutes. Capsaicin will be removed and the area re-kindled 40 min later by application of a thermode at 40ºC for 5 min. Areas of hyperalgesia and allodynia and, within the area of hyperalgesia, stimulus response to von Frey filament testing, will be determined upon removal of capsaicin and after 3 re-kindlings at 40 minute intervals. Three blood samples will be obtained after the spinal oxytocin injection at the following intervals: 10, 45, 120 minutes. The blood will be drawn from the existing intravenous catheter that is in place. If the sample cannot be obtained through the intravenous catheter, the sample will be abandoned. The samples are being drawn for the analysis of systemic oxytocin after spinal administration.

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 and the FDA within 24 hrs. Peripheral oxyhemoglobin saturation, BP and HR will be measured non-invasively before and 15, 30, 60, 120, 180, 240, minutes after administering spinal oxytocin and the electrocardiogram will be monitored continuously for the duration of the study.

A monitor tracing from the EKG will be recorded at 15 minute intervals for the first hour then hourly until discharge. 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. A screening neurologic exam along with questioning for qualitative symptoms will be performed at 45, 90, 150, 210, 240 minutes, and 24 hours. The screening neurologic test will consist of examining both an arm and leg for deep tendon reflexes (DTR's), light touch, extension/withdrawal strength. In addition we will ask the volunteer about

other symptoms to include sedation, anxiety, nausea, GI, bladder, dizziness, extremity weakness, or any other symptom which was not specifically asked. Any symptom proffered will be categorized on a scale of the volunteer's choosing.

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. If BP elevation is judged to be due to pain following spinal oxytocin injection, we will treat pain first (at the volunteer's request) and only treat BP elevations if the volunteer has sufficient analgesia (he does not desire additional analgesia). 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 glycopyrolate 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.

Completion of Day 2 Assessment

1. Vital signs are within normal limits blood pressure (systolic 90-140 mmHg; diastolic 50-90 mmHg) resting heart rate 45-100 beats per minute) without medication
2. He or she can ambulate without difficulty (no motor, balance affects).
3. Someone other than the volunteer will take the volunteer home (requirement for participation).
4. Volunteer promises to stay home where he or she can be contacted by telephone (requirement for participation).

We will instruct the volunteer to call us, providing beeper and telephone numbers of the research nurse and the investigators should he have any questions or problems. The research nurse will call the volunteer at 6 hours and 12 hours post-spinal oxytocin injection inquiring about any perceived problems.

Study Visit 3: Approximately 24 hours after the spinal oxytocin injection the subject will report to the CRU to have a blood sample drawn for a Basic Metabolic Panel to assess serum sodium levels.

RISKS

Risks of spinal needle insertion include pain on insertion, local bruising or soreness, post-dural puncture headache, and permanent nerve injury. Participants in this study will be informed regarding these risks. Local anesthesia will be used to prevent pain on needle insertion, and a small (#25 or #27) Whitacre tip needle used to minimize local trauma and risk of headache. We routinely telephone all volunteers daily for 5 days. Participants will also be contacted weekly for 1 month, then approximately 6 months after the study to inquire regarding local discomfort or headache. There is a risk (less than 1%) of developing a headache from the spinal needles. If a subject develops a headache that does not go away and is bothersome, offer to perform an epidural blood patch. This procedure is 95% effective in treating the headache. Rarely (5 out of 100 times) is a 2nd blood patch needed. Other risks of the epidural blood patch are the same as the spinal. The risk of paralysis, infection or nerve damage from a spinal procedure is extremely rare, less than one chance in 20,000. The numbing medicine that we put under your skin right before the spinal needle is inserted may cause you to have a brief period (about 5 seconds) of mild discomfort. We have had no local discomfort lasting more than 2 weeks and only 1 headache in 438 subjects that required epidural blood patch treatment.

Risks of intrathecal oxytocin include unforeseen toxicity. This drug will be studied under FDA oversight and approval. The safety of spinal oxytocin has been extensively examined in animals, with no evidence of any nerve damage or other lasting effects, the FDA has approved this investigational study for humans, nearly 1000 humans have received spinal oxytocin in China without reported problems, and we did not observe toxicity in the recently completed Phase 1 study at this institution. Although this all suggests that it is safe to administer oxytocin, the exact risks are unknown. We will monitor subjects for many theoretical problems such as level of sleepiness, changes in blood pressure, heart rate and breathing. We will treat any side effects that the subject may experience if the changes are enough to concern us.

HUMAN SUBJECTS

All studies in volunteers will be performed in the CRU, which includes monitoring and resuscitation equipment and trained nursing support staff, or in in-patient settings of the Wake Forest Baptist Medical Center. All studies will be approved by the IRB and written informed consent obtained. The Project Investigators have all performed similar studies in volunteers and patients. The purpose of the study and all risks will be discussed with each volunteer, and all questions will be answered prior to obtaining written informed consent. Risks to be discussed include discomfort with needle and catheter insertion, risk of postdural puncture headache and spinal needle insertion, and potential period of pain if the study drugs are ineffective. The most likely bothersome risk to volunteers will be that of headache from i.t. needle insertion. We will discuss options for headache treatment including oral caffeine and epidural blood patch at the time of original consent and again should a headache occur. These will be provided at no charge to the volunteer, paid for by departmental funds. All data acquired will remain confidential with no reference to individuals in publications.

Data Safety Monitoring Plan

This Phase II trial will be performed with a data safety monitoring committee (Laura Dean, MD and Francis Walker, M.D.) who is not involved in the study. Data and adverse events will be reported to these individuals quarterly. Adverse events will also be reported to the IRB and the FDA (since trials are being conducted under an IND). Serious adverse events will be reported to all of these groups and the data safety monitoring individuals within 24 hr and the trials halted until feedback is obtained from each. Data and any adverse events will be monitored and reviewed by the PI after treatment of each subject.

Minority, Gender, and Children Participation

Based on previous experience with volunteer studies, women will represent 40-60% of participants in the proposed studies. However, as seen in the table below from the past cycle with volunteers, it is unlikely that more than 20% of participants will be minorities. There is no reason to suspect a difference among races in response to these analgesic compounds, so from a scientific viewpoint this will not be detrimental. We will recruit form our IRB approved Database (BG 05-468). Children under age 18 are not included in these protocols because this protocol involves the use of experimental compounds and we believe they should be first characterized in adults. Furthermore, the study of experimental pain in children volunteers is

difficult ethically. For these reasons we anticipate studying approximately 6-7% of our subjects under age 21, and none under age 18.

Volunteer Payment

Participants will be paid a total of $500. 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.

Placement of IV: $25

If IV placement unsuccessful, the volunteer is paid and the study abandoned.

Placement of Spinal: $125 and study drug administration

If the 1st spinal is attempted but proves unsuccessful, the volunteer is paid $100 and the study abandoned,

Application of capsaicin cream: $25 for each application (total $50)

Completion of entire study: $300

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

Long-term Follow-up

Volunteers will be contacted daily for 5 days, weekly for 1 month, and at 6 months after the study and questioned about neurologic symptoms, symptoms of post-dural puncture headache (spinal headache) or other complaints.

STATISTICAL RATIONALE

Primary outcome measure will be the area of hyperalgesia after the first skin heating following topical capsaicin.

Group comparison: Areas of hyperalgesia after the first skin heating following topical capsaicin will be compared among the three groups using one-way ANOVA.

Power analysis: Based on previously published work by us with this model in which the intrathecal placebo group had an area of hyperalgesia of 62 ± 12 cm^2, a group size of 12 will allow us to observe a difference among groups of 15 cm^2 with $\alpha=0.05$ and $1-\beta=0.80$. A 25% reduction in area of hyperalgesia is considered by us and other groups to be clinically predictive

of reduction in hyperalgesia and in spontaneous pain in patients with neuropathic pain with the same intervention.