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.

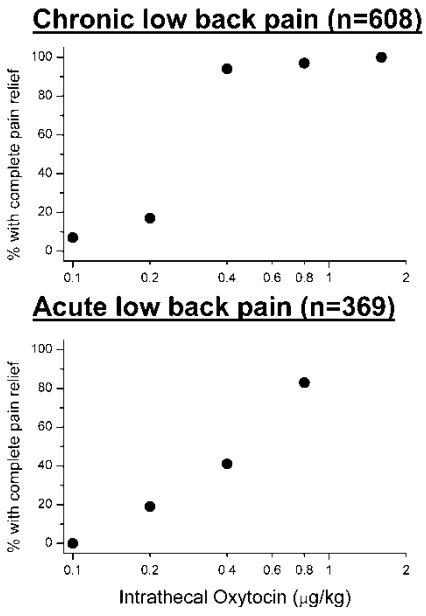

Figure 1. Percentage of patients with complete pain relief from intrathecal injection of oxytocin.

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

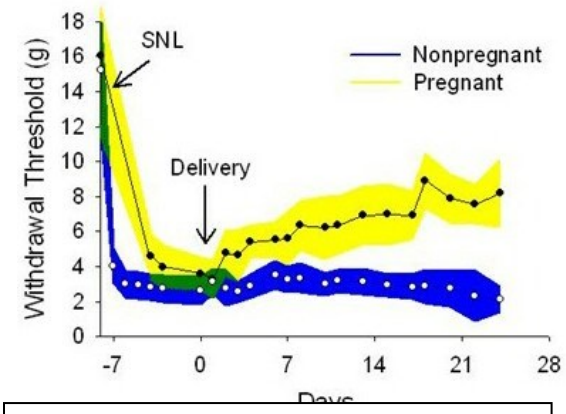

Figure 2. Withdrawal threshold drops similarly after spinal nerve ligation (SNL, a model of neuropathic pain) in pregnant and nonpregnant animals, but this hypersensitivity is alleviated after delivery. Colored areas are 95% CIs.

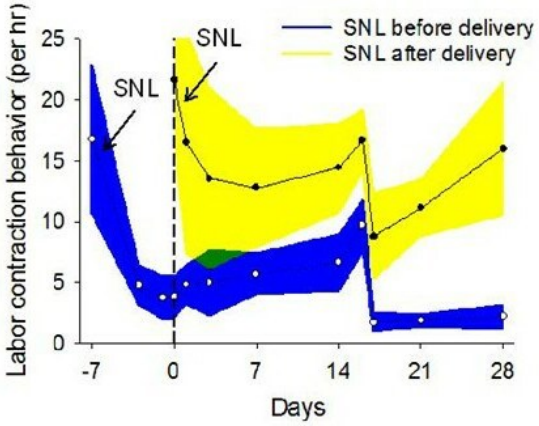

Figure 3. In animals with SNL performed during pregnancy, withdrawal threshold drops abruptly when pups are weaned at 17 days, but when SNL is done on the day of delivery, withdrawal threshold never changes from control.

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 to acutely relieve chronic neuropathic pain. For these future studies, we will use a cross over, controlled and blinded study of intrathecal oxytocin in patients with neuropathic pain below the umbilicus, with primary outcome being visual analog score for pain and secondary outcomes being areas of increased pain sensation to a normally painful stimulus (hyperalgesia) and pain sensation to what previously was an innocuous stimulus (allodynia).

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Protocol

Efficacy of Intrathecal Oxytocin in Patients with Neuropathic Pain

GOAL: Determine the effect of intrathecal oxytocin on pain and areas and intensity of hyperalgesia and allodynia in patients with chronic neuropathic pain.

METHODS: Equal numbers of healthy men and women will be recruited. On a day at least 3 days before study, patients will come to the CTSI Clinical Research Unit (CRU) to review the informed consent, confirm eligibility criteria, and be trained to rate pain in response to heat applied to an area of normal skin without ongoing pain using a Peltier controlled thermode, and to rate pain in response to 225 mN von Frey filament testing within the area of hyperalgesia, if present.

Subjects will come to the CTSI CRU at least 3 days later and be randomized into one of 2 groups of 20 subjects each to receive, in a double-blind manner, intrathecal saline or intrathecal oxytocin, 100 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. Following subject rating of their ongoing pain and mapping of areas of allodynia and hyperalgesia, as well as rating of pain by a 225 mN von Frey filament applied in the area of hyperalgesia, an intrathecal injection of the study solution in a 3 ml volume will be performed via a #27 or #25 Whitacre needle inserted in a lower lumbar interspace. Subjects will rate their ongoing pain and that from the von Frey filament in the area of hyperalgesia, and we will map the areas of hyperalgesia and allodynia at 30, 60, 90, 120, 180, and 240 min after injection. Subjects will return a second time to the CTSI CRU at least 1 week after the first study session and the same procedures will be performed, except that they will receive the other intrathecal study drug solution (e.g., if they received saline on study session 1, they will receive oxytocin on study session 2).

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 spinal needle to inject study solution. In our experience, this occurs in less than 10% of participants. Subjects will be recruited at Wake Forest School of Medicine pain clinics, and Orthopaedic and Podiatry

clinics at the Wake Forest Davie County facility. Subjects will also be recruited from our existing database BG 05-468 and we will also utilize the Clinical Translational Science Institute's (CTSI) Enterprise Data Warehouse (EDW) and approved advertising in the community for potential subjects.

STUDY DESIGN

Inclusion Criteria: We request permission to study up to 44 subjects so that we will have 40 evaluable subjects.

1. Male or female ≥ 18 and < 70 years of age, with a BMI <40.

2. Presence of neuropathic pain for > 6 months, with primary pain area below the umbilicus. We will focus recruitment efforts on low back pain with a neuropathic component to the lower extremities, but will accept other causes of neuropathic pain.

3. 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.

4. Blood pressure (systolic 90-150 mmHg; diastolic 50-90 mmHg) resting heart rate 45-100 beats per minute.

5. All subjects must be vaccinated against SARS-CoV-2, with proof via vaccination card or the NC HHS form.

Exclusion Criteria:

1. Hypersensitivity, allergy, or significant reaction to any ingredient of the study drug, lidocaine, or latex.

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 and adenomyosis as reported on history), 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. Pain treatment that includes spinal cord stimulators, chronic intrathecal drug therapy, or oral opioid treatment for > 3 months at a current dose of > 100 mg morphine per day or equivalent.

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 100 μg oxytocin on the first study visit, with the other on the second.

Study Visit 1: The participant will report to the CTSI 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. Participants will be trained to estimate pain quantitatively using a 2 cm^2 peltier controlled thermode (TSA®) applied to an area of normal skin outside the area of ongoing pain. Typically, the participant is exposed to temperatures between 38^0 and 51^0 C using a random staircase method.

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

Study Visit 2

On the second visit, the participant will report to the CTSI CRU in the morning. 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, 100 μg 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 Compounding, Winston-Salem, NC 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. Ongoing pain, pain to 225 mN von Frey filament application in the area of hyperalgesia, and areas of hyperalgesia and allodynia will be determined at 30, 60, 90, 120, 180, and 240 min after spinal injection. Blood samples (5 ml) will be obtained at 10, 20, 40, and 75 min after spinal injection by aspiration from the indwelling catheter, plasma will be prepared from the samples and frozen for subsequent analysis for oxytocin content. If blood cannot be obtained from the catheter we will not collect samples.

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 ECG 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, and 240 minutes. 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 20% of those on admission to the CRU
2. He or she can ambulate without difficulty (no motor, balance affects).
3. Someone other than the subject will take the subject home (requirement for participation).
4. Subject promises to stay home where he or she can be contacted by telephone (requirement for participation).

We will instruct the subject 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 subject at 6 hours and 12 hours post-spinal study drug injection inquiring about any perceived problems.

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

Study Visit 4: At least one week after Study Visit 2 the subject will return to the CTSI CRU for intrathecal injection of the cross over study solution. All procedures will be the same as Study Visit 2.

Study Visit 5: Approximately 24 hours after the spinal oxytocin injection the subject will report to the CTSI 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 subjects 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 and in a study of over 90 subjects undergoing hip replacement surgery 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.

Previous Experience in the CRU

These investigators have, under NIH grant GM48085, regularly performed intrathecal injection studies with novel drugs for intrathecal delivery in healthy volunteers and in patients since 1990. Drugs studied include clonidine, fentanyl, neostigmine, adenosine, ketorolac and oxytocin. In addition, we have studied drugs approved for intrathecal delivery (morphine and bupivacaine) in the CRU. The most common adverse effect from these studies has been mild, transient backache and headache. Persistent headache requiring blood patch has occurred in <1% of subjects, likely reflecting the use of very small gauge (#27), pencil point spinal needles. Drug related adverse effects have included hypotension and bradycardia (clonidine), nausea (fentanyl, neostigmine), mild respiratory depression (fentanyl), transient back and leg pain (adenosine) and one episode of hypotension and bradycardia consistent with a vaso-vagal response to injection (oxytocin). The IRB and FDA recognizes that there are potential risks to these studies and has supported performance of this research in the CRU with the presence of an anesthesiologist and nurse,

appropriate monitoring, intravenous access, and immediate access to appropriate drugs for treatment.

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. 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.

Subject Payment

Participants will be paid a total of $800 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.

Placement of IV on each study day: $25

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

Placement of Spinal on each study day: $100 and study drug administration

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

- Completion of study Visit 2 (first spinal injection): $150

- Completion of study Visit 4: (second spinal injection): $250
- Completion of entire study (completion of all study related procedures): $150

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

Long-term Follow-up

Subjects 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. We will also collect a verbal pain score during these follow ups.

STATISTICAL RATIONALE

Primary outcome measure will be ongoing pain by visual analog scale.
Group comparison: Pain will be compared using a repeated measures ANCOVA.
Power analysis: Based on previously published work by us with this model in which the patient population has an ongoing pain level of 4.3 ± 2.0 and a reduction in pain 60 min after injection of 0.9 ± 1.5, a group size of 40 will allow us to observe a difference between placebo and treatment of 0.7 with $\alpha=0.05$ and $1-\beta=0.80$.