Kisspeptin Physiology in Patients with Hyperprolactinemia

NCT02956447

Study Protocol and Statistical Analysis Plan

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BROAD GOALS

The overall goals of this protocol are to stimulate gonadotropin secretion and folliculogenesis using exogenous kisspeptin administration.

SPECIFIC AIMS

Aim 1: To stimulate gonadotropin secretion using exogenous kisspeptin.
Hypothesis: The inhibitory effect of hyperprolactinemia on GnRH secretion is mediated through kisspeptin. Therefore, kisspeptin administration to hyperprolactinemic women intolerant to conventional therapies will result in an increase in GnRH-induced gonadotropin release.

Aim 2: To stimulate folliculogenesis using exogenous kisspeptin.
Hypothesis: The suppression of folliculogenesis that occurs in hyperprolactinemia can be overcome by kisspeptin administration. Therefore, kisspeptin administration to hyperprolactinemic women intolerant to conventional therapies will result in mature follicle development.

SUBJECT SELECTION

This study will consist of two cohorts, one for each specific aim.

Inclusion/Exclusion Criteria

Inclusion:

- Female, Ages 18-45,
- Confirmed diagnosis by elevated levels of prolactin measured via blood test
- Physical Examination:
    - normal blood pressure, (systolic BP < 140 mm Hg, diastolic < 90 mm Hg).
- Laboratory Studies:
    - white blood cell, platelet counts, and TSH between 90% of the lower limit and 110% of the upper limit of the reference range,
    - hemoglobin no less than 0.5 gm/dL below the lower limit of the reference range for normal women,
    - BUN, creatinine, AST, ALT not elevated,
    - negative serum hCG pregnancy test at the time of screening (additional urine pregnancy test will be conducted prior to drug administration).
- Willing to complete a dopamine agonist washout period 6±2 weeks prior to each of the frequent blood sampling visits (Cohort 1) or 6±2 weeks prior to completing relevant screening labs until the end of the study (Cohort 2). The washout period will last at least 4 weeks.
- Not using oral contraceptives or willing to complete an oral contraceptive washout period 8 weeks prior to each of the frequent blood sampling visits (Cohort 1) or 8 weeks prior to completing relevant screening labs until the end of the study (Cohort 2).

Exclusion:

- Patients with a macroprolactinoma confirmed on MRI Imaging
- Current or recent use of a medication that, in the opinion of a study investigator, can modulate the reproductive axis

- History of a medication reaction requiring emergency medical care
- A medical condition that, in the opinion of a study investigator, would likely interfere with participation in/completion of the protocol
- Excessive alcohol consumption (>10 drinks/week) and/or use of illicit drugs
- Pregnant or trying to become pregnant
- Patients with a history of bilateral oophorectomy (ovaries were removed)
- Breast feeding

STUDY PROCEDURES

General Outline of Protocol

The goal of these studies is to assess the role of kisspeptin in GnRH and gonadotropin secretion as well as folliculogenesis in patients with hyperprolactinemia. GnRH cannot be measured in the peripheral circulation, and so LH will be measured as a surrogate marker of GnRH secretion. LH is secreted by the pituitary in direct response to GnRH, and there is a well-established one-to-one concordance between GnRH and LH pulses. [1,2]

Medication Washout

Cohort 2 subjects on dopamine agonists will be asked to complete a washout period 6±2 weeks prior to completing relevant screening labs and lasting until the end of the study. Cohort 2 subjects on oral contraceptive pills will be asked to undergo a washout period of 8 weeks prior to completing relevant screening labs and lasting until the end of the study. Subjects will be asked to discuss the washout with their prescribing physician prior to beginning washout. Any subjects who experience intolerable side effects as a result of medication washout will end participation in the study and be instructed to follow recommendations by their prescribing physician regarding resuming any medications.

Screening Visit

The screening visit will include a complete history and physical examination and blood tests (listed below). The total volume of blood drawn during screening will not exceed 18mL.

Screening laboratory tests

- Progesterone, Estradiol, Prolactin
- White blood cell count, platelet count, TSH, hemoglobin, BUN, creatinine, AST, ALT
- Serum hCG pregnancy test

Additional blood draws may be scheduled to repeat the progesterone and estradiol tests from the screening visit. This is to determine the phase of the menstrual cycle prior to subject's study visits. The volume of blood drawn is approximately 5 mL. The total volume of blood drawn will not exceed PHRC guidelines.

The screening history may be obtained by phone. Documentation of a physical examination performed by a non-study physician within the previous 12 months may be used in lieu of an in-person physical examination; in this case, a physical examination will also be performed by a study physician at Visit 1. To obtain physical examination results performed off-site, subjects will be given an MGH "authorization for disclosure of medical information from another facility" form. If a physical examination is performed solely for the purposes of this study, study funds will pay for the cost of the physical examination. Remote screening laboratory studies will be performed at MGH on a blood sample shipped by the subject or at a local laboratory site (e.g. LabCorp,

Quest, etc.); study funds will pay for all phlebotomy and shipping costs. Laboratory studies already performed within the previous 6 months will be in lieu of repeating screening studies unless abnormal.

Cohort 1 Protocol: Gonadotropin secretion (N=10)
Subjects will complete four outpatient visits. Procedures for each visit are described below:

Screening Visit: Described above

If eligibility criteria are all met, a member of the study team will call the subject and schedule them for two visits at the Clinical Research Center.

As mentioned above, we will ask subjects being treated with dopamine agonists to complete a washout period prior to the frequent blood sampling visit(s). The washout period will last at least 4 weeks.

Visit 1

- Admission to the Clinical Research Center (CRC)
- q10 minute blood sampling (3 mL q 10 min) x 12 hours without intervention to assess endogenous LH pulsatility. All samples will be assayed for LH. Progesterone (P4) will be measured on the first sample. FSH, prolactin, and estradiol (E2) will be assayed from pools of the frequent sampling studies.[3,4]

Visit 2 (within 22 days of Visit 1)

- Admission to the Clinical Research Center (CRC)
- q10 minute sampling (3 mL q 10 min) x 12 hours
- Administration of 10 boluses of kisspeptin 0.313 µg/kg IV every hour beginning at hour 1 of the study. All samples will be assayed for LH. Progesterone (P4) will be measured on the first sample. FSH, prolactin, and estradiol (E2) will be assayed from pools of the frequent sampling studies.[3,4]
- Administration of one bolus of GnRH 0.075 ug/kg IV at hour 11 of the study.

Visit 3: Final follow-up study visit
This visit will occur within a month after subjects have completed their Visit 2. The total blood drawn during this visit is 13mL. If any test result is abnormal, the subject will be followed until screening tests (CBC, BUN, creatinine, liver function studies) return to the ranges specified in eligibility criteria. If hemoglobin <10 g/dL then subjects will be provided with a one month supply of iron pills.

Cohort 2 Protocol: Folliculogenesis (N=5)
Subjects will complete 9 outpatient visits. Procedures for each visit are described below:

Screening Visit: Described above
If eligibility criteria are all met, a member of the study team will contact the subject to schedule Visit 1.

Visit 1: Day 1 of kisspeptin administration

- Participants will undergo a pelvic ultrasound to assess presence or absence of follicular growth. It is at investigator discretion to halt participation of any subject with evidence of follicular growth from the Visit 1 ultrasound.

- Participants will come to the Reproductive Endocrine Unit, or the CRC, where a study physician will initiate the administration of kisspeptin.
    - Option 1: Kisspeptin 0.313 – 13.19 µg/kg SC q60 – q240 minutes will be administered subcutaneously.
    - Option 2: Kisspeptin 0.313 µg/kg IV q 90 minutes will be administered using a closed IV system.
- Up to 10 blood samples will be drawn during the visit. One or more 3mL samples will be collected just before the initial kisspeptin is delivered and additional 3mL samples will be collected after the bolus is delivered, over the course of the next 80 minutes (maximum total blood collection volume of 30mL) to define the LH response to the kisspeptin. All blood samples will be assayed for LH. P4 will be measured on the first sample, and prolactin will be measured on both the first and final samples. Pooled samples will be used for measurements of FSH and E2. Time of day of sample collection will be recorded.
- Vital signs will be collected at least once during the visit.

Visits 2-7: Daily blood sampling (days 2-7 of kisspeptin administration)

- The GnRH-induced gonadotropin responses to a single bolus of kisspeptin (administered via pump(s)) will be monitored with up to 10 blood samples: One or more 3mL samples will be collected just before a kisspeptin bolus is delivered and additional 3mL samples will be collected after the bolus is delivered, over the course of the next 80 minutes, to define the LH response to the kisspeptin bolus. Maximum blood volume collected each day is 30mL . Sample collection will begin at the same time of the day ± 3 hours from the time collection began at Visit 1.
- All blood samples will be assayed for LH. P4 will be measured on the first sample, and prolactin will be measured on the first and final samples. Pooled samples will be used for measurements of FSH and E2. Time of day of sample collection will be recorded.
- Pelvic ultrasounds will be performed at Visit 3 or Visit 4 to monitor follicular growth. This ultrasound is intended to ensure that the rate of follicular growth and number of follicles do not indicate hyperstimulation, which is considered extremely unlikely to occur, based on prior data of kisspeptin administration in this dose range.
- Vital signs will be collected at least once during each daily visit.

Visit 8: Final Visit (day 8 of kisspeptin administration)

- At least 30 minutes after kisspeptin administration ends, participants will undergo final hormonal measurements: LH, FSH, E2, P4, prolactin; and safety laboratory studies: AST, ALT, BUN, Cr, hemoglobin, white blood cell, platelet counts, and TSH. The total blood drawn during this visit will not exceed 20mL.
    - The study team will contact participants if any safety value is outside the desired range (as stated in eligibility criteria) and arrange appropriate follow-up as needed. If hemoglobin <10 g/dL then subjects will be provided with a one-month supply of iron pills.
- Pelvic ultrasound will be performed to assess follicular growth.

Blood Draws:

Cohort 1: The total volume of blood drawn during screening will not exceed 18ml. The progesterone and estradiol tests drawn at screening may be repeated; approximately 5mL will be drawn. The study subjects will undergo two 12 hour sampling studies on two separate days, approximately 220mL will be drawn for each study. The total volume of blood drawn for final

follow-up is approximately 13mL. The total volume of blood drawn over the course of the protocol is within PHRC guidelines and will not exceed 550 mL.

Cohort 2:
The total volume of blood drawn during screening is 13-18mL. Subjects will then undergo daily monitoring blood draws over 7 days. Total volume of blood drawn during Visits 1-7 will not exceed 30mL each day. The total volume of blood drawn during Visit 8 will not exceed 20mL. The total volume of blood drawn over the course of the protocol is within the PHRC guidelines and will not exceed 550 mL.

If a study subject wants to participate in both Cohort 1 & Cohort 2, there will be a minimum of 8 weeks between study participation in Cohort 1 and participation in Cohort 2, to conform with PHRC guidelines.

Justification of doses:

Kisspeptin IV: The dose of kisspeptin 112-121 0.313 µg/kg (0.24 nmol/kg) IV x1 is the same dose used in the PI's studies in healthy men and women and is the dose predominantly used in the PI's studies of men with IHH. This dose was chosen based on the first reported use of kisspeptin in humans, which showed that doses smaller than this dose (using a molar equivalent) elicited a smaller response (rise in LH), whereas larger doses did not elicit a larger response.[5] In other words, a dose of 0.24 nmol/kg was the smallest dose to elicit a maximal response. A more recent report of dose-response studies with kisspeptin made similar findings.[6] The LH amplitudes of kisspeptin-induced-GnRH- induced LH pulses are comparable to the LH amplitudes observed endogenously during the follicular and luteal phases of the female menstrual cycle have LH amplitudes comparable to (follicular phase: kisspeptin induced LH amp 1.1 ± 1.0 vs. endogenous LH amp 1.2 ± 0.5 mIU/mL; mid-luteal phase: kisspeptin induced LH amp 4.0 ± 2.8 vs. endogenous LH amp 3.0 ± 1.5 mIU/mL)[7] Moreover, in men, the range of LH amplitudes observed for kisspeptin-induced-GnRH-induced pulses overlaps the range of amplitudes that occurs endogenously(1.6 –10.9 and 0.5–7.8 mIU/ml, respectively).[8] Thus, kisspeptin 0.24 nmol/kg (0.31 µg/kg) is a physiologic dose in healthy adults.

Kisspeptin SC: The dose range of kisspeptin 112-121 is 0.313 µg/kg (0.24 nmol/kg) – 13.19 µg/kg (10.1 nmol/kg). A range of doses is provided to account for a differential response of SC administration as compared to IV administration, as confirmed by the PI's other work.

GnRH: Long experience with GnRH has shown that a physiologic dose of GnRH is 25-75 ng/kg.[9] To ensure that a response to GnRH is seen, the dose of GnRH 75 ng/kg IV x1 is used in these protocols. The dose used to achieve pituitary priming is GnRH 25 ng/kg SC q2h x6d, which has been shown to successfully achieve priming.[10]
Data suggests there is no interaction between kisspeptin administration and changes in prolactin levels in humans. Data recently published in Clinical Endocrinology show that prolactin levels are not significantly changed immediately following kisspeptin-54 injections (6.4 nmol/kg) in healthy women.[11] Our group has observed similar short term results after administration of single boluses of kisspeptin-10 (0.313 µg/kg; 0.24 nmol/kg) in healthy men and men with IHH. Long-term follow up data collected by our group in healthy men, cycling women, and postmenopausal women, as well as men and women with IHH, also show that prolactin levels do not significantly change after administration of boluses or infusions of kisspeptin-10.

Cohort 1 CRC Visits

Females will undergo a pregnancy test at screening and again at admission prior to receiving kisspeptin. Subjects will receive isovolumetric replacement with saline during blood sampling. Kisspeptin will be administered by a CRC RN. Vital signs (HR, BP, RR, and T) will be measured every 1-4 hours throughout the frequent sampling portions of the protocol and every 10 minutes for up to 30 minutes after kisspeptin administration.

Termination criteria will include: 1) blood pressure > 180 mmHg systolic or >105 mmHg diastolic at any point during the protocol; 2) development or suspicion of an allergic or serious adverse reaction; 3) positive pregnancy test; 4) temperature ≥101.5°F at any point in the study. Any subject who develops high blood pressure or an allergic reaction will receive follow-up inpatient medical care until the issue is resolved, with subsequent outpatient and/or phone follow-up as needed. The CRC is fully equipped to handle medical emergencies, with trained staff, a bag-valve mask in the room, a crash cart on the floor, and rapid access to a physician code team.

Cohort 2 daily blood sampling visits
Females will undergo a pregnancy test at screening and again prior to receiving kisspeptin. Kisspeptin will be administered via a pump(s). Vital signs (HR, BP, RR, and T) will be measured at least once during each daily blood sampling visit.

Termination criteria will include: 1) blood pressure > 180 mmHg systolic or >105 mmHg diastolic at any point during the protocol; 2) development or suspicion of an allergic or serious adverse reaction; 3) positive pregnancy test; 4) temperature ≥101.5°F at any point in the study. Any subject who develops high blood pressure or an allergic reaction will receive follow-up inpatient medical care until the issue is resolved, with subsequent outpatient and/or phone follow-up as needed.

BIOSTATISTICAL ANALYSIS

Assays
Given the long-term ongoing nature of this study, the assay methodologies employed to characterize the biochemical profile (both reproductive and metabolic) will be based on standard accepted methods as established by the Reproductive Endocrine Reference Laboratory. As such these methods may include immunoassays, measurement using standard laboratory platforms, radioimmunoassays, and mass spectroscopy. We anticipate that these methods will continue to evolve as the diagnostics field changes.

Cohort 1 (Specific Aim 1)
Population Size
Each patient will serve as her own control. The primary outcome will be the number of LH pulses on the first biochemical assessment (no kisspeptin) compared to the second assessment (+kisspeptin). Many studies in the literature have reported a decreased LH pulse frequency in hyperprolactinemia with one study (using q 10 minute sampling) documenting approximately 2.25 pulses / 12 h in hyperprolactinemic individuals.[12] During the second admission, by administering 10 boluses of kisspeptin, 10 LH pulses should be observed. For the purposes of these calculations however, 8 pulses will be conservatively estimated because some of those pulses will coincide with/conceal endogenous pulses. For a difference of 8 and an SD of 6.8, 10 subjects will need to be studied before and after kisspeptin to achieve 90% power at alpha = 0.05.

Data Analysis and Interpretation

LH pulses will be identified using a modified version of the Santen and Bardin method of pulse detection which has been validated *in vivo*.[3,4] The primary outcome will be change in the frequency of LH pulses in response to kisspeptin administration. The hypothesis will be accepted if the change in LH frequency on kisspeptin is higher during intermittent kisspeptin administration (second assessment) than in the absence of kisspeptin administration. Mean LH level and LH pulse amplitude will also be calculated and used in secondary analyses.

Cohort 2 (Specific Aim 2)

Data analysis and interpretation

The primary endpoint will be evidence of follicular growth from Visit 1 to Visit 8. We anticipate that to detect a mean difference of 7.8mm in maximum follicle size and a standard deviation of 4.4, 5 subjects will need to be studied to achieve 80% power at alpha = 0.05.

POTENTIAL BENEFITS

Participants with hyperprolactinemia will not benefit directly from this study. The only benefit from participating in this study comes from the altruistic contribution to furthering our understanding of the biology of human reproduction.

XI. REFERENCES

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