“Metabolism of 0.35mg Norethindrone vs 5mg Norethindrone Acetate”

NCT05294341

7/09/2024

Background:

Norethindrone has an elimination half-life of about 8 hours. Norethindrone acetate has an initial disappearance with half life of 7.5 minutes, with subsequent elimination half-life of 51.5 hours. Singh et. al. demonstrated that equilibrium was reached between NET and NETA 24-48 hours. Prior to 24 hours, norethindrone predominates. After 72 hours, norethindrone acetate predominates. This study suggested that the prolonged half life of norethindrone acetate contributes to prolonged action of norethindrone acetate

Study Objectives:

Specific Aim 1: To assess the relative bioavailability of norethindrone in individuals taking 5mg norethindrone acetate vs 0.35mg norethindrone

Specific Aim 2: To assess the maximum and minimum norethindrone concentrations in individuals taking 5mg norethindrone acetate vs 0.35mg norethindrone

Specific Aim 3: To assess the differences in serum levels of estradiol, LH, FSH, and progesterone levels in individuals taking 0.35mg norethindrone vs 5mg norethindrone acetate

Hypothesis: The relative bioavailability of norethindrone will be comparable in individuals taking 5mg norethindrone acetate vs 0.35mg norethindrone.

Design:
We will collect patient serum samples over the course of a week to assess for amount of norethindrone in their blood. We will also examine estradiol, follicle stimulating hormone, luteinizing hormone, and progesterone levels at various timepoints in the study. We will have patients fill out a symptom diary to document bleeding profile and side effects from their medications. This symptom diary will also have patients demonstrate satisfaction on their therapy.

Subjects in each group will be assigned either 0.35mg norethindrone or 5 mg norethindrone acetate as their initial therapy. They will start taking either medication on day 1 of their menstrual cycle. Serum levels will be drawn at 8 am for 7 days. On day 8 they will stop taking norethindrone, and will have their serum levels drawn hourly for 8 hours. They will then undergo a 1 month washout. They will do the above with the opposite therapy.

The blood draws will take place in the Clinical Research Center on the 4th floor. The samples will be stored in the Clinical Research Center freezers. Dr. Jeffrey Neighbors will then analyze the amount of norethindrone there is in each assay.

Methods:
This study was conducted at the Milton S. Hershey Medical Center College of Medicine, and Penn State Health's Outpatient

Obstetrics and Gynecology clinic. Institutional Review Board (IRB) approval was obtained through the Penn State College of

Medicine IRB.. Study participants provided written informed consent as part of their enrollment into the study.

Study Participants

Women aged 18-55 years old, with menstrual length cycles 24-34 days, , using reliable non-hormonal contraception

(permanent contraception or copper IUD), and the ability to follow-up, were eligible for the study. Participants were

excluded if they were pregnant within the last 3 months had a history of irregular menstrual cycles, on hormonal

contraception, hormone replacement therapy, or hormonal treatment of infertility in the last 3 months, history of

hysterectomy, liver disease, kidney disease, breast cancer, venous thromboembolism. . Potential subjects were identified directly in the clinical setting during clinic visits by the PI and co-I or identified by medical records review of individuals who had undergone bilateral tubal sterilization procedures for undesired fertility in the last 6 months. They were called by the co-I to assess interest in the study, and times were arranged to consent the subject in person.

Study Treatment

NETA and NET tablets were packaged and supplied by the Investigational Drug Service (IDS) Pharmacy. Paper scripts for each treatment were provided to the subjects, and were filled and distributed by the IDS.

Study Design

This is a cross-sectional pilot study examining serial plasma concentrations of norethindrone, luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone in individuals taking either NET or NETA.

There was no randomization process. The study was divided into 2 arms with the same protocol and procedures for each arm. The first half of subjects recruited were assigned to NET as their initial intervention. The second half of subjects were assigned to NETA as their initial intervention. Neither subjects nor study coordinators were blinded to medication assignment. However, the laboratory personnel were blinded to the medications assigned in each sample analyzed.

Subjects were instructed to begin taking their assigned medication on days 1-3 of their menstrual cycle. They received text message reminders to take their medication between 6:30-8:00AM daily. Within one hour of self-administering the medication, they presented to Penn State Health Milton S. Hershey Medical Center, for phlebotomy to measure concentrations of plasma norethindrone. This was repeated for 7 days. On day 8 subjects stopped taking their medication, and initiated pharmacokinetic (PK) testing with hourly blood drawls for 8 hours to assess their norethindrone levels. They then underwent a 1-month washout, after which the above was repeated with the opposite medication. Additionally, we drew phlebotomy for estradiol, follicle stimulating hormone, luteinizing hormone, and progesterone levels at days 1, 8, and 21 of each phase. Blood samples were centrifuged immediately after collection, and plasma was collected and stored in a -40C freezer until ready for analysis.

Days 1-8 of both phases, subjects completed a symptom diary to document bleeding profile and side effects. The symptom diary included: irregular bleeding, changes in menstrual cycle, changes in vaginal discharge, nausea, vomiting, headaches, weight gain, mood changes, breast pain, abdominal pain, abdominal bloating, pelvic pain, swelling of the legs, and other. A validated bleeding quantification tool was also included. . At the end of day 8, subjects completed a survey that assessed the following: subject satisfaction with their medication, whether they experienced any side effects, whether they experienced any improvement in preexisting symptoms, and whether the subjects had additional feedback. The Symptom Diary was filled out on days 1-8 for each phase, and returned to the site at the Day 8 PK visit of phase 2. At the end of the study, each participant completed an additional survey about the feasibility of study, including taking their medication at the same time every day, presenting for daily blood draws at the same time every day, and obtaining hourly blood draws on day 8.

Study Endpoints and Measurements

Analysis of norethindrone in plasma

Chemicals

Norethindrone was purchased from Sigma-Aldrich (St. Louis, MO) and 13C2-norethindrone was purchased from Cambridge Isotope Laboratories (Tewksbury, MA).

Analysis of plasma drug levels

Plasma concentrations of norethindrone were determined using mass spectrometry. Standard curves were constructed by plotting the ratio of the analyte peak area to internal standard peak area versus analyte concentration. The standard working solution (4 mL) and internal standard (4 mL) were spiked into control plasma (10 mL), and after vortexing, acetonitrile/H2O/formic acid (90/10/0.1) (22 mL) was added to extract the analytes from plasma. Proteins were precipitated by vortexing with subsequent centrifugation at 8,765 g for 10 minutes at 4°C. The supernatant was taken and loaded to the HPLC/MS/MS system, with final concentrations of 1 ng/mL to 1000 ng/mL for norethindrone.

For norethindrone analysis, after spiking 4 mL 13C2-norethindrone into 200 mL plasma, 600 mL acetonitrile/H2O/formic

acid (90/10/0.1) was added for extraction. The supernatant was dried and the residue was reconstituted by 40 mL acetonitrile/H2O/formic acid (90/10/0.1) before loading onto HPLC-MS-MS system. The calculated concentrations from the standard curves were divided by 5 to reflect the in vivo levels of norethindrone in plasma.

Norethindrone in plasma was analyzed using a Sciex QTRAP 6500+ mass spectrometry coupled with a Sciex EXion HPLC separation system. A 1.7 mm Acquity UPLC BEH C18 analytical column (2.1 x 100 mm, Waters, Ireland) was used. The gradient elution was conducted using a flow rate of 0.4 mL/min with the following conditions: initial at 70% mobile phase B (acetonitrile) and 30% mobile phase A (0.1% formic acid in water), followed by a linear gradient to 90% mobile phase B in 1 minute, and kept at 90% mobile phase B for 3 additional minutes to flush the column before bringing back to initial conditions to equilibrate the column.

The Sciex QTrap 6500+ mass spectrometer was equipped with an electrospray ionization probe operated in positive mode. The decluster potential (DP) was 131 V; the entrance potential (EP) was 10 V, the collision energy (CE) was 25 V, while the collision cell exit potential (CXP) was 12 V. The curtain gas (CUR) was 35 L/h, the collision gas (CAD) was medium. The ionSpray voltage was 5500 V, the temperature was 600 °C, gas 1 was 15 L/h, and gas 2 was 15 L/h.

The multiple reaction monitoring mode (MRM) was used to analyze and quantify norethindrone and 13C2-norethindrone, with the transitions of 299 > 231 for norethindrone, 301 > 231 for 13C2-norethindrone. All peaks were integrated and quantified by Sciex OS 1.5 software.

Statistical Analysis Plan

Serum Levels of norethindrone were averaged across all subjects on days 1 through 7. This was done for individuals taking both 5mg norethindrone acetate and 0.35mg norethindrone. Standard deviations for each of these averages were calculated. Serum levels of norethindrone were then averaged across all subjects on hours 0-8 on day 8 of the study. This was done for individuals taking both 5mg norethidnrone acetate and 0.35mg norethindrone. Standard deviations for each of these averages were calculated.