University of Pennsylvania Perelman School of Medicine

Multimodal Imaging of Progesterone/Neurosteroid Effects in Nicotine Addiction

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CONFIDENTIAL

Abstract

Nicotine addiction is a major public health concern. The purpose of this study is to examine whether short-term progesterone administration reduces withdrawal symptoms, cigarette craving, and smoking behavior in female and male smokers who abstain from smoking for three days. In addition, we seek to determine whether progesterone administration alters brain gamma-aminobutryic acid (GABA) levels and whether neurotransmitter changes are related to changes in withdrawal, craving, and smoking behavior

Background

Sex differences in response to nicotine

Recently there has been an increased awareness of how sex affects disease states and drug responses, including response to drugs of abuse, in humans. For nicotine response, several recent studies suggest differences between men and women. Women, without previous discrimination training, were less able to discriminate the effect of nicotine nasal spray on a broad range of doses, compared with men (Perkins, 1995). Women may also be less able to titrate their smoking in response to changes in cigarette brand or with prior nicotine administration, compared with men (Perkins et al., 1992). In addition, among those quitting smoking, men but not women, self-administer nicotine nasal spray more than placebo suggesting sex differences in reinforcing effects of nicotine (Perkins, 1996). Men and women may also differ in suppression of nicotine withdrawal symptoms by nicotine. Others have observed that women had less suppression of cigarette withdrawal symptoms with 2 mg nicotine polacrilex, compared with men (Hatsukami et al., 1995). Further supporting this observation, several studies reported that female smokers might benefit from nicotine replacement treatment for smoking cessation less than men (Benowitz and Hatsukami, 1998; Gritz et al., 1996; Wetter et al., 1999). Since nicotine seems to be less reinforcing in women, it has been suggested that non-nicotine smoking stimuli may be more reinforcing in women, compared with men (Perkins, 1996; Perkins et al., 2002). This idea, if proven, may have important treatment implications. Altogether, these studies suggest that women, compared with men, may be less sensitive to the subjective and reinforcing effects of nicotine.

Menstrual cycle effects in nicotine dependence

The menstrual phase effects on nicotine response were investigated in a recent study. No menstrual cycle phase effects were observed on the subjective and physiological response to nicotine nasal spray (Marks et al., 1999). The main limitation of this study was a large dropout rate, 12 completers out of 35 enrolled, which limited the power of the study. The tendency of women to be less able to discriminate the effects of nicotine nasal spray, compared to men, may also limit the sensitivity of the study to detect changes in nicotine response. Further studies are needed to determine whether phase of menstrual cycle affects the subjective or reinforcing effects of nicotine.

In contrast to nicotine response, two recent studies reported significant menstrual phase effects in response to cocaine and amphetamines. Sofuoglu and colleagues (1999) investigated sex and menstrual phase differences in response to single and repeated doses of smoked cocaine in humans. They found significant sex differences on subjective ratings of cocaine effects, with women reporting lower ratings compared with men. However, when the phase of the menstrual cycle was taken into consideration, a clearer picture emerged. Women in the luteal phase reported diminished ratings of subjective effects of cocaine, compared with both women in the follicular phase of the menstrual cycle and men. The average plasma estradiol and progesterone concentrations during the luteal phase were 156 pg/ml and 6.1 ng/ml, respectively. For the follicular phase, the corresponding values were 61 pg/ml and 0.43 ng/ml, respectively. These higher progesterone levels in luteal phase, compared to the follicular phase, suggest that progesterone may contribute to the diminished response to stimulants during the luteal phase. Similar menstrual phase effects were also reported in response to amphetamines in healthy women (Justice and de Wit, 1999). Women had diminished subjective response to oral amphetamines during the luteal phase, compared with the follicular phase. Whether or not the luteal phase also attenuates the drug use behavior has not been investigated. These studies are relevant for nicotine considering the common neural substrate, the dopaminergic system, proposed to mediate the reinforcing effects of stimulants (Pich et al., 1997).

Menstrual cycle phase may also affect the severity of tobacco withdrawal symptoms. One study reported greater withdrawal symptoms in women who abstained from smoking during the late luteal phase than either women in the mid-cycle or men (Craig et al., 1992). A number of other studies also reported increased intensity of tobacco withdrawal symptoms from abstinence during the late luteal phase (4-5 days before menses) (Allen et al., 2000; DeBon et al., 1995; O'Hara et al., 1989; Pomerleau et al., 1992; Sofuoglu et al., 2001), another study showed increased withdrawal during the luteal phase (Perkins et al., 2000). Although other studies showed no menstrual cycle effects on tobacco withdrawal severity, (Allen et al., 1999; Pomerleau et al., 1994b) menstrual cycle phase may also affect smoking behavior in women. Around menses and during the late luteal phase, increased smoking behavior was reported in some (DeBon et al., 1995; Mello et al., 1987; Pomerleau et al., 1992; Snively et al., 2000; Steinberg and Cherek, 1989) but not in other studies (Allen et al., 1996; Pomerleau et al., 1994b). Altogether, these results suggest that menstrual phase may affect nicotine withdrawal symptoms and perhaps smoking behavior in female smokers. The main limitation of these studies examining menstrual cycle phase effects is the use of various and sometimes imprecise terms and methods to define the phases of the menstrual cycle which makes it difficult to compare the findings from these studies.

The increased severity of tobacco withdrawal symptoms and the smoking behavior in late luteal phase or menses, coincides with rapid decrease in plasma estradiol and progesterone levels suggesting hormonal influence on the severity of withdrawal symptoms and possibly on smoking behavior. Unfortunately, little is known about the hormonal mechanisms that mediate the menstrual cycle phase effects on tobacco withdrawal symptoms or smoking behavior. To determine the hormonal mechanisms of menstrual cycle effects, a reasonable approach is to administer sex hormones, estradiol and progesterone, to normally menstruating women and characterize the dose-related effects of these hormones on selected outcomes. The main difficulty with this approach is the interaction of exogenous sex hormones with endogenous estradiol and progesterone that fluctuate during the menstrual cycle. That is, estradiol or progesterone treatment given during the follicular or luteal phase is expected to have different effects because the endogenous levels of estradiol and progesterone highly vary in these phases. This problem is in large part resolved by administering exogenous hormones in the early to mid follicular phase when endogenous levels of estradiol and progesterone are at their nadir.

<u>Early follicular phase to study the effects of sex hormones</u>
The early follicular phase of the menstrual cycle is defined as the first 7 days after the onset of menses. While the endogenous estradiol levels are low and remain stable, below 85 pg/ml, during the early follicular phase, their levels start to increase, up to 400 pg/ml, during the late follicular phase, 8-13 days after the onset of menses. The progesterone levels remain low throughout the follicular phase, below 1.1 ng/ml (Speroff et al., 2007). The low and stable levels of estradiol and progesterone in the early follicular phase minimize the interaction between the endogenous and exogenous sex hormones. In addition, disruption of the menstrual cycle or withdrawal bleeding are unlikely to occur since the endogenous estradiol levels are minimal. Several studies have used early follicular phase to administer exogenous estradiol or progesterone to normally menstruating women. In a series of studies, Tan et al. administered estradiol and progesterone for six days to women in the early follicular phase during two consecutive cycles (Tan et al., 1996; 1997b) and progesterone to healthy males (Tan et al., 1997a). More recently Justice and deWitt (2000b) used a similar approach to examine the effects of estradiol patch treatment on amphetamine response during the early follicular phase of two consecutive menstrual cycles. In our pilot studies, we examined the feasibility of administering progesterone treatment during the early follicular phase of the menstrual cycle to female smokers (Sofuoglu et al., 2001) and female cocaine users (Sofuoglu et al., 2002a). These results suggest that the low and stable endogenous estradiol and progesterone levels during the early follicular phase provide a unique opportunity to administer exogenous sex hormones to normally menstruating women.

1 Study Objectives

1.1 Overall Objectives

The overarching goal of this study is to examine progesterone's effects on brain GABA concentrations and to determine whether these neurochemical changes are related to behavioral changes/experiences typically observed in individuals with nicotine addiction who are undergoing short-term abstinence.

1.2 Primary Outcome Variable(s)

Occipital cortex GABA concentrations pre and post progesterone and placebo administration. GABA concentrations will be obtained using the non-invasive neuroimaging method of proton magnetic resonance spectroscopy (1H-MRS) both before and after progesterone and placebo administration and smoking abstinence. We will also administer an fMRI task called the N-back and measure BOLD signal and an fMRI task called the cue task, in addition to a resting state task where participants are asked to focus on a crosshair symbol. In total subjects will undergo 3 combined 1H-MRS and fMRI scans during each study phase over a period of approximately 1 month for men and approximately 2 to 4 months for women.

1.3 Secondary outcome variable(s)

Withdrawal symptoms will be measured using the Nicotine Withdrawal Symptoms Checklist (NWSC). Craving will be assessed using the Brief Questionnaire on Smoking Urges (BQSU) and mood will be assessed with the Profile of Mood States (POMS) and the Positive and Negative Affect Scales (PANAS), the State-Trait Anxiety Inventory (STAI), and the Visual Analogue Scale (VAS). The above secondary outcome measures will be assessed at baseline, on the day of scans, and at each abstinence check-in with research staff. The Center for Epidemiologic Studies Depression scale (CES-D), adverse events form (AEF), Adverse Childhood Experiences (ACE) questionnaire, and Tiffany QSU will also be included as secondary outcome variables. Premenstrual syndrome symptoms will be assessed daily throughout all study phases using the Daily Record of Severity of Problems (DRSP).

2 Study Design

2.1 General Design

This project seeks to determine, during a 3-day period of abstinence, whether progesterone 200 mg/d administration for four days modulates cortical amino acid concentration as measured using proton magnetic resonance spectroscopy (1H-MRS), reduces withdrawal symptoms and cigarette craving and alters smoking behavior in male and female smokers. This study utilizes a double-blind, randomized placebo-controlled, cross-over design. We will recruit 75 female and 75 male smokers who are between the ages of 18 and 50. Subjects will be recruited from flyers posted around campus, direct home mailings, collaborations with our Penn TTURC investigators, the Penn Data Store service to identify potential subjects to be sent a recruitment email and eligibility survey, and paid advertisements. Study subjects will be screened first by phone by a member of the research staff who can gauge whether the caller is likely to meet study criteria. Callers will be informed that this is not a treatment study and all subjects who report that they are treatment seeking will be given the number for our TTURC colleagues who are in charge of treatment studies being conducted at that time.

Callers who appear to meet criteria and express an interest in the study will be invited to undergo an in-office screening which will require approximately 2 hours. Participants who report for an in-office screening will first be read a verbal consent form for pre-screening procedures. Pre-screening procedures include breath carbon monoxide level and urine drug screen. Participants have the right to decline pre-screening procedures and complete these procedures after full consent. Should the participant not be qualified based on pre-screening procedures, the records will be destroyed and they will be dismissed from the study visit. If the participants are eligible based on pre-screening procedures, full, written ICF will be reviewed and signed with the study CRC. After giving full written informed consent, subjects will undergo a structured psychiatric evaluation using the SCID-IV Non-Patient and several behavioral ratings and questionnaires concerning personal history. The Fagerstrom scale and urine cotinine level will be used to assess degree of nicotine addiction. Women must complete the Daily Record of Severity of Problems (DRSP) daily throughout all study test phases and during washout to assess presence of premenstrual symptoms. Women who have not had a menstrual cycle for 6 months or more at screening will not be asked to complete the DRSP during study participation because they do not experience mood symptoms revolving around a menstrual cycle. Women will also complete a pregnancy test at screening.

Upon completion of the screening visit, subjects will be scheduled to undergo their first ad-lib smoking and combined 1H-MRS and fMRI scan sessions. Women experiencing a menstrual cycle will be scanned during the early-mid follicular phase of the menstrual cycle for two cycles. Menstrual cycle phase will be determined by report of last menstrual period, typical menstrual cycle length, and report of onset of menstrual flow. Once onset of flow has been reported, best effort will be made to schedule women for their first smoking and scan sessions between Day 2 and Day 8 with first day of menstrual flow designated as Day 1. Men and non-menstruating women will be scheduled to undergo 2 testing sessions, approximately one- to two-weeks apart.

To obtain a baseline assessment of smoking behavior, subjects will present for their first smoking session prior to their first 1H-MRS and fMRI scan session. This smoking session will be considered Test Day 1. A blood sample and urine sample for cotinine levels will be completed at this visit. They will be seated comfortably and given a series of ratings to assess mood, anxiety, nicotine craving, and withdrawal. Ad-lib smoking sessions will last 2 hours and subjects will smoke their cigarette brand of choice. Subjects will smoke through a plastic cigarette holder fitted to the filter end of a cigarette connected to a smoking topography device (CreSS from Plowshare Technologies). This device will assess the smoking topography (e.g., puff frequency, puff volume, puff duration, inter-puff interval, maximum flow rate, and inter-cigarette interval). Upon completion of the smoking session, subjects will complete additional questionnaires. CO level will also be assessed pre- and post- smoking session.

In preparation for their first scan day, subjects are instructed to smoke as usual but to make sure that they have at least one cigarette upon awakening and to have their last cigarette right before entering the Stellar-Chance Laboratories where the Center for Magnetic Resonance and Optical Imaging (CMROI) is located. This final cigarette will insure that subjects are not in nicotine withdrawal at their baseline scan. CO level will be assessed immediately following the completion of the participant's final cigarette. Subjects may be asked to abstain from consuming alcohol the evening before scan sessions at the principal investigator's discretion.

Prior to undergoing scans on Test Day 2, subjects will complete behavioral questionnaires to assess mood, anxiety, nicotine craving, and withdrawal. Subjects will also provide blood samples. They will then undergo a baseline 1H-MRS and fMRI scan, which requires approximately 90 minutes. They will be taken out of the scanner and asked to self-administer either the oral micronized progesterone (Prometrium) 200 mg or a look-alike placebo capsule. Immediately after, subjects will be given a meal, as food enhances the absorption of progesterone. Neither the subject nor the Study Coordinator will know to which group the subject is assigned. After a time period up to 3 hours the subject will undergo a second 1H-MRS and fMRI scan which requires approximately 90 minutes. The subject will complete additional questionnaires and another blood draw, and they will then be discharged from Test Day 2.

Prior to the participant's first dose of study medication, a supplemental informed consent form pertaining to the study medication and placebo will be reviewed with the participant and signed by a physician listed on this study protocol.

All subjects will be met by a member of the study staff twice on Test Days 3 and 4 (morning and afternoon) and once (morning) on Day 5 prior to the chronic treatment scan session to assess abstinence (expired breath CO less than 10 ppm or a continually decreasing CO level during days 3-5 that is greater than 10) and to obtain behavioral measures. At the morning visits on Test Days 3 and 4, participants will self-administer the progesterone or placebo medication.

On Test Day 5, subjects are asked to present to the PCWBW to self-administer their final dose of medication. Breakfast will be provided to them. Abstinence will be assessed via CO level prior to the post-treatment scan. The study staff will then escort the participant to CMROI for their post-treatment 1H-MRS and fMRI scan which requires approximately 90 minutes. Upon completion of the second scan session, subjects will be escorted again to the testing laboratory where they will undergo a second 2-hour ad lib smoking session. Expired breath CO level will be assessed again prior to the smoking session to ensure continued abstinence prior to this session. Blood samples and a urine sample will be obtained prior to the subject's smoking session on Test Day 5.

All subjects complete behavioral ratings to assess mood, anxiety, nicotine craving and withdrawal as well as expired breath CO level at all study visits.

Upon completion of Test Day 5, all male subjects will then undergo an approximately one- to two-week wash-out and all menstruating female subjects will undergo an approximately 1-3 month wash-out. Subjects will then cross over to the other treatment condition. Upon crossover, the study procedures described above with be repeated. Women will be asked to provide a urine sample for a pregnancy test before continuing to the second paradigm.

The primary investigator and research staff will unblind themselves to the drug treatment that was administered in each study phase upon each participant's completion or discharge from the study. The research staff will unblind the participant by reviewing lab work that reveals progesterone levels at each phase of the study. Research staff will also reach out to IDS to confirm the nature of each participant's randomization schedule.

NOTE: GABA data will not be obtained at the 3T MRI scanner because proton magnetic resonance spectroscopy is not reliable on the 3-tesla magnet. Only the fMRI tasks and resting state will be completed at 3T. Retrieval of GABA data will be resumed when the new 7T MRI scanner at Stellar Chance is obtained. The estimated date for when the study will be able to resume at 7T is in the summer of 2017.

2.2 1H-MRS Methods

All experiments will be performed on a Siemens 3T PRISMA system with any of the Seimens product coils suitable for brain studies and CAMRIS approved RF pulse sequence adapted from that Greutter's group at the University of Minnesota (Terpstra et al., 2002). Side-to-side head movements will be minimized by placing foam pads between the volunteer's ears and the volume coil. GABA data will be acquired using the MEGAPRESS pulse sequence and from 15 to 40 ml volumes with echo times of 70 ms and recycle delays of 3-5 seconds to obtain the best signal to noise. Two thousand and forty-eight complex points will be sampled with a receiver bandwidth at 2 kHz. Prior to FFT, J-difference time-domain spectra will be apodized with a 3.5-Hz exponential function. Selective inversions will be achieved with 15.64-20 ms pulses at the 1.91-ppm C-3 resonance of GABA on odd-numbered acquisitions and at a position symmetric about the water resonance on even-numbered acquisitions in order to reduce baseline artifact. CHESS pulse will be applied for water suppression.

2.3 Medication Administration

Subjects will be prescribed progesterone 200 mg capsules or a look alike placebo by the study PI (Epperson, M.D.) or another doctor listed on this protocol. Subjects will take 200 mg of progesterone or a placebo pill daily for 4 days.

2.4 Compensation

If the participant completes all parts of the study, is on time for appointments, takes their medication, and does not smoke during the abstinence period, total payment for participation is $1,350. Pre- and peri-menopausal women who are asked to complete the DRSP can receive up to $1,450 in total compensation. Participants will also be provided with lunch on Test Day 2 and breakfast on Test Day 5 of both Phases 1 and 2.

The breakdown of subject payment is as follows.

Visit Name	Amount	Notes
Screening	\$50	Contingent upon eligibility
Phases 1 & 2		
Test Day 1	\$50	
Test Day 2	\$250	
Test Day 3	AM - \$12.50, PM - \$12.50	If participant: • Self-reports a break in abstinence, • Their breath CO level is not continuously decreasing • Or within a non-smoking range (10 ppm) at abstinence check-in (beyond what could be attributed to contamination from environmental factors) The participant will receive \$5.00 instead of \$12.50 for that check-in visit. If participant's tests and self-report indicate abstinence prior to subsequent check-ins, payment will return to full \$12.50 amount.
Test Day 4	AM - \$12.50, PM - \$12.50	See above.
Test Day 5	\$150	Breath CO prior to scan and smoking session will confirm continued abstinence. If participant: • Self-reports a break in abstinence, • Their breath CO level is not continuously decreasing • Or within a non-smoking range (10 ppm) at abstinence check-in (beyond what could be attributed to contamination from environmental factors) The participant will receive \$10 for post-treatment smoking session and \$25 for post-treatment scan (\$35 total).

If participants complete all of Test Phase 1, they will receive an additional payment of $100. If participants continue on to complete all procedures in Test Phase 2, they will receive an additional payment of $200.

Females participating in the study who are given the DRSP will be given a $100 payment for completing a DRSP for the duration of study participation (beginning Phase 1, Test day 1 and ending Phase 2, Test Day 5).

2.5 Study duration

We anticipate completion of recruitment within 5 years of study inception. Each subject will spend approximately 2-4 months completing all study related procedures.

3 Subject Selection and Withdrawal

3.1 Target Population

We will recruit 75 male and 75 female with nicotine addiction for at least 1 year. We anticipate a dropout rate of at least 4 subjects per group and have planned accordingly. Thus, we anticipate being able to enroll a socioeconomically and racial/ethnically diverse group such that the composition of the proposed study population will reflect the greater Philadelphia referral base. The projected composition from Philadelphia is African American 42.58%, Hispanic 8.5%, Asian 4.42%, Other 2.04% and White, not of Hispanic Origin, 42.46%. The size of our expected sample may allow us to detect effects due to ethnicity or minority status.

3.2 Accrual

For our primary outcome variable--GABA concentration: Based upon pilot data from our group examining the acute effects of progesterone 800 mg administration on healthy human subjects, we anticipate that an acute dose of progesterone will alter GABA concentrations by approximately 15% on average. We will conservatively estimate a 10% reduction in occipital GABA concentration. In order to detect a 10% change in GABA concentrations with a power of 80% and an alpha of 0.05 we will need to study 28 male and 28 female smokers. As this study can be time consuming, we will expect a 10-15% drop out rate between study sessions. This study's inclusion/exclusion criterion may also account for some drop-off after the initial screening visit. Thus, we will recruit 75 male and 75 female subjects for a total of 150 subjects.

We anticipate being able to recruit this number of subjects within a 24-month period of time based upon our discussions with TTURC collaborators. In fact, many of the subjects waiting to participate in their treatment study could participate in our neuroimaging prior.

3.3 Key inclusion criteria

1. Female and male smokers, aged 18 to 50 years;
2. History of smoking greater than or equal to 10 cigarettes daily for the past year, as per self-report (periods of smoking abstinence within the last year will be exclusionary at PI discretion);
3. Not seeking treatment at the time of the study for nicotine dependence;
4. Have a FNTQ score of at least 3 and CO level greater than or equal to 11ppm;
5. In good health as verified by self-reported medical history;
6. Clean urine drug screen, (marijuana and nicotine are permissible);
7. For women, not pregnant as determined by pregnancy screening or breast feeding

3.4 Key exclusion criteria

1. History of major medical illnesses; including liver diseases, abnormal vaginal bleeding, suspected or known malignancy, thrombophlebitis, deep vein thrombosis, pulmonary embolus, clotting or bleeding disorders, heart disease, diabetes, history of stroke or other medical conditions that the physician investigator deems as contraindicated for the patient to be in the study, as determined by participant self-report;
2. Regular use of psychotropic medication (antidepressants, antipsychotics, or anxiolytics), as per self-report, and recent (within previous year) psychiatric diagnosis and treatment for Axis I disorders including major depression, bipolar affective disorder, generalized anxiety disorder, post-traumatic stress disorder and panic disorder, as determined by clinician administered SCID interview;
3. Lifetime history of schizophrenia or other psychotic disorder, as per SCID interview
4. Lifetime substance dependence disorder, excepting nicotine, alcohol and marijuana, as per SCID interview
5. Substance use disorders within the previous 2 years, excepting nicotine, as per SCID interview
6. Regular use of any other tobacco products than cigarettes, including smokeless tobacco and nicotine products, as per self-report;
7. Known allergy to progesterone or peanuts (vehicle for micronized progesterone), as per self-report.

3.5 Subject Recruitment

Subjects will be recruited by word of mouth, referral by other colleagues who study nicotine addiction, direct mailings, flyers/brochures, the Penn Data Store service to identify potential subjects to be sent a recruitment email and eligibility survey, and paid advertisements.

4 Analysis Plan

4.1 Statistical Analysis

The independent variables will be treatment and sex. For treatment there will be 2 levels: progesterone (200 mg/day) or placebo. Sex will have 2 levels: male and female.

The primary dependent variables will be 1) GABA concentrations (see note in Study Design), 2) measures of smoking behavior: alveolar carbon monoxide, and smoking topography variables (number of cigarettes, puff number, puff volume, puff duration and inter-puff interval), and 3) tobacco withdrawal symptoms measured with nicotine withdrawal symptom checklist (NWSC).

The secondary dependent variables will include progesterone and estradiol levels, Profile of Mood States (POMS), Positive and Negative Affect Scales (PANAS), Nicotine Withdrawal Symptoms Checklist (NWSC), Brief Questionnaire on Smoking Urges (BQSU), State-Trait Anxiety Inventory (STAI), Visual Analogue Scale (VAS), Center for Epidemiologic Studies Depression scale (CES-D), Tiffany QSU, Daily Record of Severity of Problems (DRSP), adverse events form (AEF), and Daily Record of Severity of Problems (DRSP).

The analysis will include only data from subjects who complete the study, were compliant with the study medication and abstinent from smoking. Based on our previous experience with similar studies, the dropouts are expected to occur early in study participation. For this reason we are not proposing detailed methods to handle missing data which are more appropriate for outpatient clinical trials. However, if we have a dropout rate over 20 percent, we will conduct a sensitivity analysis (Verbeke and Molenberghs, 2000) to examine the influence of missing data on our statistical model.

The primary analysis of the data will be done with analysis of variance. In this model, the main effect of treatment (progesterone versus placebo), sex (male vs. female) and the interaction between treatment and sex will be analyzed. Standard F-tests from the analysis of variance will be used to assess significance of treatment effects on GABA concentrations. Post hoc analyses will be conducted where significant differences are found, using Bonferroni adjustments and/or Dunnett multiple-comparison tests to preserve the overall experiment wise error rate in the case of multiple testing. Secondary dependent variables including progesterone and estradiol levels, POMS, PANAS/NWSC/BQSU,CES-D, Tiffany QSU, AEF, and DRSP, will be examined in an analogous fashion to the primary outcome variable.

In addition, regression analysis will be conducted using plasma progesterone concentrations as a predictor of GABA concentrations, tobacco withdrawal symptoms, and craving. Likewise, GABA concentrations will be used as a predictor of tobacco withdrawal symptoms, nicotine craving, smoking topology, nicotine liking and mood.

4.2 Rationale for sample size

NOTE: As per updated funding requirements in 2015 and as approved by the study Sponsor, we have increased recruitment numbers to 150 participants to account for a high number of incomplete and ineligible participants. The information below pertains to the original grant submission and original recruitment numbers.

Sample size is estimated for the 3 primary outcome measures: GABA concentrations, tobacco withdrawal symptoms, nicotine craving, smoking topology and subjective effects of nicotine.

GABA concentration: Based upon pilot data from our group examining the acute effects of progesterone 600 mg administration on healthy human subjects, we anticipate that an acute dose of progesterone will alter GABA concentrations by approximately 15% on average. We will conservatively estimate a 10% reduction in occipital GABA concentration. In order to detect a 10% change in GABA concentrations with a power of 80 % and an alpha of 0.05 we will need to study 28 male and 28 female smokers. As this study can be time consuming, we will expect a 10-15% drop out rate between study sessions. Thus, we will recruit 32 male and 32 female subjects for a total of 64 subjects

Smoking behavior: Power analysis for the study is based on progesterone effects on smoking self-administration session, from Sofuoglu et al, 2001. In that study, under placebo treatment, an average of 3.8 out of 10 reinforcers were earned for puffs of cigarettes. Under progesterone treatment, the average exchange rate was 2.3. With a SD of difference 2.7, an alpha level of 0.05, an unpaired t-test indicates a power of 80 percent for 28 male and 28 female subjects.

Subjective effects of smoking: Similar to the smoking behavior, power analysis for subjective effects will be based on our pilot data. One of the Nicotine Effects Questionnaire items, Good Effects was used for power analysis. Under placebo treatment the average rating following sample smoking was 65.9. The corresponding value was 51.1 under progesterone treatment. With a SD of difference 18.9, an alpha level of 0.05, an unpaired t-test indicates a power of 97 percent for 28 female and 28 male subjects.

Tobacco Withdrawal Symptoms: From our pilot data, one of the tobacco withdrawal symptoms, Craving for cigarettes, was used for power analysis. Under placebo treatment the average rating for this item was 3.1 following sample smoking. The corresponding value was 2.5 under progesterone treatment. With a SD of difference 0.79, an alpha level of 0.05, an unpaired t-test indicates a power close to 90 percent for 28 female and 28 male subjects.

5 Safety and Adverse Events

5.1 Potential Risks

Risks of Progesterone: Progesterone administration can cause sleepiness, nausea, fatigue, and headaches in men and women, and menstrual irregularity, spotting or breakthrough vaginal bleeding, and breast tenderness in women. Use of progesterone simultaneous to use of contraceptives is highly unlikely to lead to adverse health outcomes or interfere with contraceptive efficacy. Other less common side effects include depression, blockage of blood vessels, and increased risk for heart attack or stroke. However, these side effects mostly occur after long-term use of progesterone and are unlikely to occur with 4 days of treatment. The effects of progesterone and nicotine combined are not known.

Risks of 1H-MRS Operating at 3T: To date no persistent adverse effects have been reported by facilities with magnetic field strengths at 3.0T.

> **Functional Imaging Tests:** It is possible that staring at the projected screen during tasks could result in some eyestrain, mild headache, or nausea, however this unlikely. If subjects begin to feel uncomfortable while viewing the screen during tasks, they may look away and let the research staff know of their discomfort.
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> **Flying Object Clause:** The known risks associated with this study are minimal. The greatest risk is a metallic object flying through the air toward the magnet and hitting an employee or patient. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. No metal objects are allowed to be brought into the magnet room at any time. In addition, once a patient is in the magnet, the door to the room will be closed so that no one inadvertently walks into the magnet.
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> **Experimental Device Clause:** Some of the pulse sequences and/or RF coils are not FDA approved but are considered non-significant risk investigational devices.

Pregnancy Clause: Although there are no known risks of MRI on pregnant women or a fetus, there is a possibility of yet undiscovered pregnancy related risks. Since there is no direct benefit from participating in this protocol for a pregnant woman, we will exclude pregnant women. Attestation of pregnancy suffices as evidence that the participant is not pregnant at the time of an MRI scan.

Magnetic Field Risk Statement: Because the magnetic field of the MRI scanner attracts metal, these studies will not be performed on anyone with a pacemaker or any non-removable metallic foreign objects in their body. The use of intrauterine devices (IUDs) and subdermal implants has not shown to present any risk when operating at a 3T MRI. (Zeiman and Kanal, 2007; Kaunitz. 2012; Correia, 2012).

Health Risk Statement: There are no known health risks associated with exposure to magnetic fields during an MRI. There are minimal risks from the loud noise associated with the MRI scanner and from the discomfort of lying on a hard surface. We shall provide subjects with protective earplugs as necessary and make every attempt to ensure their comfort during their time in the scanner. The MRI performed under this protocol is not for medical purposes and the images are not planned to be interpreted by a physician

Clinician Administered Interview: Subjects will be administered the SCID-NP at the screening visit. The risk associated with administering this type of interview is minimal; however, subjects may find it upsetting to discover that they have a psychiatric diagnosis of which they were unaware. As research staff associated with this study have training regarding how to screen for the presence of psychiatric disorders, it is unlikely that a subject with a psychiatric diagnosis would not be identified during the initial phone screen and thus present for the in-office screen and SCID interview. If this were to occur however, the study PI would be available to speak at length with the individual in question and make appropriate recommendations and referrals for follow up care.

Blood Drawing: Blood drawing may cause fainting, bruising, discomfort, or rarely, infection. A total of one half of a cup of blood will be drawn over the course of the entire study. There will be a total of 8 blood draws during the study: four during Phase 1 and four during Phase 2.

Smoking Abstinence: Stopping smoking causes a nicotine withdrawal. Nicotine is the main ingredient in cigarettes. Nicotine withdrawal can cause changes in mood such as sadness, irritability, nervousness, and anxiety. During the smoking abstinence portion of the study, we will help him or her by providing contingency management. This plan provides daily support during the contingency management Test Days of Phases 1 and 2 and involves daily check in visits to measure carbon monoxide levels. During these visits, subjects are encouraged to talk about their cravings, mood symptoms, and any difficulties that they have been experiencing. Subjects will be provided with ideas to help reduce cigarette cravings. We will ask subjects to alert us right away if they are feeling very depressed or down. If anyone feels this way after office hours, they will be instructed to please call 911 and ask for assistance.

For Women of Childbearing Age: Since this research may have bad effects on a fetus and should not be done during pregnancy, it is necessary that a pregnancy test be done first. In order for female subjects to participate in this study, they must report that to their knowledge they are not pregnant at the present time. They will also agree to avoid becoming pregnant (i.e., to use an acceptable method of contraceptive such as condoms, and to take precautions against becoming pregnant) during the study. We will do a pregnancy test during the screening visit and before subjects start Phase 1 and Phase 2. Since this research may have bad effects on a newborn, female subjects agree not to breastfeed during the study participation.

Genetic Testing: In the laboratory, genetics samples will be labeled with a number only. Subject name or any other identifying information will not be attached to the samples in the research laboratory. This measure is taken to protect confidentiality, in addition to the following specific measures:

a) The genetic testing of all samples will be used for research purposes only. No results of genetic testing from this study will appear in any subject's medical record.
b) Genetic test results will not be made available to subjects, to their doctors, or to other clinicians or any other clinical staff.
c) To protect confidentiality as much as possible, no computer records will be created that could be used to identify a subject's genetic or medical information individually. Thus, even if a "hacker" breaks into the laboratory computer system, there will be no information stored there that can identify any individual subject.
d) Information about your genes will only be stored in Dr. Epperson's laboratory, using procedures described above to protect your confidentiality, unless the information has become completely stripped of information that could identify an individual subject.

In our experience at a previous academic institution, in which many hundreds of samples have been collected, no outside agency has ever tried to gain access to any research subject's genetics samples. Dr. Epperson believes that the risk of this happening to any sample collected as part of this study is extremely small.

The goal of the genetics testing for this study is exploratory, not predictive: we seek to examine the interaction between genetic makeup and nicotine addiction.

5.2 Potential Study Benefits

There are no direct benefits for subjects in this study. Their participation may benefit individuals with nicotine addiction if these data indicate that progesterone has benefits on withdrawal symptoms, cravings and smoking behavior.

5.3 Risk/Benefit Assessment

This study represents more than minimal risk to the subject as individuals will undergo neuroimaging and treatment with progesterone which they may not have otherwise taken. Should this study show that progesterone administration improves outcomes of interest and are correlated with changes in brain GABA and glutamate concentrations, we will have additional evidence that this hormone and neurotransmitter system may be targets for the treatment of nicotine addiction.

5.4 Data Safety and Monitoring

<u>Reporting of AEs and SAEs</u>
Dr. Epperson, the study PI, is required by the study sponsor, the National Institute on Drug Abuse, to have a Data and Safety Monitoring Plan (DSMP). That plan is briefly detailed below. The full DSMP is attached to this protocol submission.

<u>Reporting of IRB Actions to NIDA</u>
Should the Penn IRB take action regarding this protocol, the PI and Penn IRB officials will notify the projects program officer within 48 business hours.

<u>Report of Changes or Amendments to the Protocol</u>
Any change that is recommended by the IRB out of concerns for patient safety will be reported to NIDA when the change has been approved by the Penn IRB.

<u>Trial Stopping Rules</u>
If there is a SAE that is a direct result of study participation that results in loss of life, serious illness or morbidity, the trial will be halted.

<u>Disclosure of Any Conflict of Interest to the DSM</u>

Faculty are required to report relationships with outside entities, in particular those involving financial remuneration, as potential conflicts of interest to the University of Pennsylvania School of Medicine on a yearly basis.

5.5 Resources Necessary for Human Research Protection

Dr. Epperson, the study Principal Investigator, has more than 15 years of experience conducting translational neuroscience research in human subjects focusing on the impact of sex hormones and neurosteroids on behavior and cognition in women. Dr. Epperson is the recipient of 3 R01 grants from the NIMH, NIDA and NIA. She is also the recipient of several K awards from the NIH. Dr. Epperson has extensive experience overseeing the conduct of research in her laboratory. She is personally responsible for all aspects of the study or delegation of duties to individuals she has trained over the years.

Males and females will be recruited for this study by flyers, and paid and unpaid advertising. Dr. Epperson also collaborates with investigators in the Transdisciplinary Tobacco Use Research Center (PI: C. Lerman) here at Penn. Individuals may be referred from TTURC studies to the one proposed herein if appropriate.

If an individual were to demonstrate the need for mental health services, they would first be referred to the PCWBW for evaluation by one of the Center's psychologists or psychiatrists. An appropriate treatment plan and/or referral would be developed as necessary. The PCWBW is housed in a large office building with 24-hour security. The Center's main offices at 3535 Market Street are comprised of 8 clinical offices, one laboratory space, copy, fax and mail room, reception area and kitchen. In addition, there is both clinical and administrative space at Pennsylvania Hospital in the same area as Maternal Fetal Medicine outpatient services.

6 Data Handling and Record Keeping

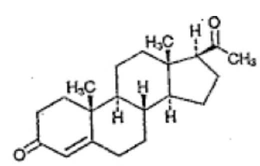
6.1 Subject Confidentiality

All subjects will be given a unique identifier to place on each of their questionnaires and behavioral ratings. Data from scan sessions will also be coded with this unique identifier. Only the project PI, the Project Supervisor and the Project Coordinator will have access to the code which will be kept in a locked file cabinet in a locked room. Five years after completion of the study, the subject code will be destroyed. Data will be destroyed 8 years after study completion.

7 Investigational Agent

7.1 Investigational Agent

- *Drug name: progesterone, Prometrium®*
- *Pharmacological class: Steroid hormone; Subclass: Progestin*
- *Structural formula (if known):*



- Prometrium capsules contain micronized progesterone that has a molecular weight of 314.47 and a molecular formula of $C_{21}H_{30}O_2$. Progesterone (pregn-4-ene-3, 20-dione) is a white or creamy white, odorless, crystalline powder practically insoluble in water, soluble in alcohol, acetone and dioxane and sparingly soluble in vegetable oils, stable in air, melting between 126° and 131° C. Progesterone is synthesized from a starting material from a plant source and is chemically identical to progesterone of human ovarian origin. Inactive ingredients for Prometrium Capsules 100 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C Yellow No.

10, and FD&C Red No. 40. Inactive ingredients for Prometrium Capsules 200 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C yellow No. 10 and FD&C Yellow No 6.

– After oral administration of progesterone as a micronized soft-gelatin capsule formulation, maximum serum concentrations were attained within 3 hours. The absolute bioavailability of micronized progesterone is not known,

- *Formulation and dose: Capsules 100 mg and 200 mg as a micronized soft gelatin capsule formulation*
- *Route of Administration: Oral PO*

Planned exposure (e.g. duration of study drug administration): Healthy, smoking adults, (age range 18-50 years) without psychiatric or substance use history will be enrolled in this study to assess the impact of progesterone administration on GABA in individuals with nicotine addiction using a 3T magnet. In addition, we will explore the impact of 4 daily doses of progesterone 200 mg on subjective reports of craving and smoking behavior in smokers who are requested to abstain from smoking for 3 days. The 4 daily doses of progesterone will be administered in 200 mg capsules on Test Days 2-5.

7.2 Overview of Previous Human Experience—Progesterone and Nicotine Effects

Sofuoglu and colleagues (2001) previously studied the direct effects of oral progesterone treatment on smoking behavior in female smokers. In each experimental session, following overnight abstinence from smoking, subjects received a single 200 mg dose of progesterone or placebo, orally. Two and a half hours after the medication treatment, subjects were assessed for subjective response to two puffs of a cigarette and then started the self-administration period, in which every 15 minutes, they had the option to exchange their earned token for two puffs of a cigarette or money. Progesterone treatment attenuated subjective rating of good effects of smoking the first cigarette and craving for cigarettes following overnight abstinence. The first cigarette following abstinence is regarded to be highly rewarding and linked to relapse in smokers trying to quit (Kenford et al., 1994). The progesterone effects on the subjective rewarding effects of smoking and craving for cigarettes suggested that this hormone may have significant effects on smoking behavior and tobacco withdrawal symptoms. More recently Sofuoglu et al. (in press) studied the effects of two doses of progesterone 200 mg/d and 400 mg/d versus placebo on measures of attention (Stroop and Digit Symbol Substitution Test) and smoking urges in 35 female and 34 male smokers. The 200 mg/d dose improved cognitive performance, while the 400 mg/d dose reduced smoking urges. All subject tolerated both dosages without serious adverse events. There were no effects of progesterone administration on mood, and only modest effects on blood pressure and heart rate, reducing heart rate in males and diastolic blood pressure in females.

The same group conducted a similar preliminary study to examine the progesterone effects on smoked cocaine responses in which cocaine-dependent women, following progesterone or placebo treatment, received three deliveries of 0.4 mg/kg smoked cocaine, 30 min apart. Progesterone treatment attenuated some of the subjective effects of cocaine further suggesting that progesterone may effect mediation of reward by psychostimulants (Sofuoglu et al., 2002a). Preclinical studies provide possible mechanisms to explain these findings. Progesterone has wide-ranging effects in the brain including the activation of GABA type A receptors (Majewska, 1992). Progesterone also affects the dopaminergic system, a neurotransmitter system, which is implicated to be an important neural substrate for both the reinforcing effects of stimulants including cocaine and nicotine and withdrawal symptoms from stimulants (Watkins et al., 2000). Preclinical studies suggest that estradiol and progesterone may have opposing effects on the dopaminergic system. While estradiol has stimulatory effects on the dopaminergic system, progesterone may have inhibitory effects (Becker and Rudnick, 1999; Dluzen and Ramirez, 1987; Fernandez-Ruiz et al., 1990; Michanek and Meyerson, 1982; Morissette and Di Paolo, 1993). These preclinical studies suggest that progesterone may potentially modulate the actions of nicotine through its effects on the dopaminergic system.

In light of these clinical findings, it is plausible that attenuation of the rewarding effects of nicotine effects by progesterone may contribute to the observed sex differences on nicotine dependence in humans. The cyclical increases in progesterone levels during the luteal phase may attenuate the effects of nicotine and may contribute to decreased sensitivity of women to the nicotine effects, compared to men. Whether these effects of progesterone administration on craving and smoking behavior are secondary to effects on dopamine or GABAergic function or both is not known. Preclinical data and our pilot data (not shown) suggests that progesterone has moderate to profound effects on GABAergic function. In some women oral progesterone administration decreased occipital cortex GABA concentrations by 11%-40%. With the research paradigm proposed in this protocol, we hope to further our understanding of how progesterone may modulate nicotine craving and smoking behavior by obtaining a central measure of the hormone's effect on brain GABA concentrations.

Interestingly, there is a growing interest in the neuroprotective effects of progesterone administration for the treatment of traumatic brain injury in both men and women. In a randomized, placebo controlled trial, Wright and colleagues (2007) gave both males and females presenting with acute brain injury a loading dose of progesterone 0.71 mg/kg intravenously followed by approximately 0.5 mg/kg per hour for 11 hours. For the following three days, subjects received progesterone 0.5 mg/kg per hour or placebo for 12 hours each day. At this rate, an average man of 170 pounds (77 kg), would have received a 54.7 mg of progesterone over the first hour followed by 424 mg over the next 11 hours. These doses were well tolerated by both male and female patients, with no serious adverse events attributable to progesterone administration. In fact, 30-day mortality rate was significantly less in those subjects randomized to progesterone compared to those randomized to placebo. This smaller study has led to a large NIH-funded Phase III clinical trial of progesterone administration for traumatic brain injury.

7.2.1 Reference to previously submitted IND application(s)

Not applicable to this application.

7.3 Overview of Preclinical Data

See FDA Labeling Attached

8 Investigational Agent: Chemistry and Manufacturing

– Prometrium capsules contain micronized progesterone, which has a molecular weight of 314.47 and a molecular formula of $C_{21}H_{30}O_2$. Progesterone (pregn-4-ene-3,20-dione) is a white or creamy white, odorless, crystalline powder practically insoluble in water, soluble in alcohol, acetone and dioxane and sparingly soluble in vegetable oils, stable in air, melting between 126° and 131° C. Progesterone is synthesized from a starting material from a plant source and is chemically identical to progesterone of human ovarian origin. Inactive ingredients for Prometrium Capsules 200 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C yellow No. 10 and FD&C Yellow No 6.

- Manufactured by: Catalent Pharma Solutions, St. Petersburg, FL 33716
- Marketed by: Abbvie, Inc, North Chicago, Illinois, 60064

8.1 General Method of Preparation and packaging

Prometrium (progesterone) is commercially available from the manufacturer.
All of the study drug will be prepared and dispensed by the Penn Investigational Drug Service (IDS). IDS backfills the Prometrium capsules with a small amount of microcrystalline cellulose (not lactose per investigator request) to ensure they seem similar to the placebo capsules.

8.2 Drug Components and Drug Product

– Progesterone is synthesized from a starting material from a plant source and is chemically identical to progesterone of human ovarian origin. Inactive ingredients for Prometrium Capsules 200 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C yellow No. 10 and FD&C Yellow No 6.

8.3 Placebo Product

Capsugel hard gelatin capsules will be used for both the placebo product and active progesterone capsules. The placebo capsules will be filled with microcrystalline cellulose.

Capsugel hard gelatin capsules are made from high-quality gelatin derived from collagen, a fibrillar protein composed of eighteen different amino acids found in connective tissues and bones. Natural bovine or porcine collagen is macerated and purified using either acids or alkalis, depending on the production process. The collagen splits hydrolytically into an unbranched amino acid chain with a molecular weight ranging from 40,000 to 100,000. This results in a high-grade, consistent granular gelatin. The high-grade gelatin used to produce Capsugel hard gelatin capsules meets all standards and regulations imposed by both the food and pharmaceutical industries.

8.4 Labeling

The drug used for this study will be placed in a bottle labeled with the subject's name, instructing them to take one 200 mg capsule by mouth on Test Days 2 - 5. The capsules will be ordered through the IDS at Penn and brought to the scan session by a member of the PCWBW research staff. The study drug will be stored at the Penn Center for Women's Behavioral Wellness and research staff will monitor and administer the study drug to the participant on each test day.

8.5 Environmental Analysis Requirements

As progesterone is a marketed medication, we believe an environmental analysis is not required.

9 Pharmacology and Toxicology

After oral administration of progesterone as a micronized soft-gelatin capsule formulation, maximum serum concentrations were attained within 3 hours. The absolute bioavailability of micronized progesterone is not known. Table 1 summarizes the mean pharmacokinetic parameters in postmenopausal women after five oral daily doses of Prometrium Capsules 100 mg.

TABLE 1. Pharmacokinetic Parameters of PROMETRIUM

Parameter	PROMETRIUM Capsules Daily Dose		
	100 mg	200 mg	300 mg
Cmax (ng/mL)	17.3 ± 21.9[a]	38.1 ± 37.8	60.6 ± 72.5
Tmax (hr)	1.5 ± 0.8	2.3 ± 1.4	1.7 ± 0.6
AUC (0-10) (ng × hr/mL)	43.3 ± 30.8	101.2 ± 66.0	175.7 ± 170.3

[a] Mean ± S.D.

Serum progesterone concentrations appeared linear and dose proportional following multiple dose administration of promethium capsules 100 mg over the dose range 100 mg/d to 300 mg/d. The pharmacokinetic parameters in male volunteers were generally consistent with hose seen in postmenopausal women. Progesterone is approximately 96% to 99% bound to serum proteins, primarily to serum albumin and transcortin. Progesterone is metabolized primarily by the liver largely to pregnanediols and pregnanolones. The glucuronide and sulfate conjugates of pregnanediol and pregnaolone are excreted in the bile and urine. Progesterone metabolites are eliminated mainly by the kidneys.

Please refer to the attached FDA-approved label from February 2010 attached for more detailed information on the pharmacodynamics, pharmacokinetics, toxicology, and safety of Prometrium.

10 Previous Human Experience with the Investigational Agent

10.1 Marketed experience (From Product Package Insert)

Progesterone is FDA approved for the prevention of endometrial hyperplasia in women undergoing estrogen treatment and the treatment of secondary amenorrhea. In a randomized, double-blind, clinical trial, 358 postmenopausal women each with an intact uterus received treatment for up to 36 months. The treatment groups were Prometrium Capsules at the dose of 200 mg/d for 12 days per 28-day cycle in combination with conjugated estrogens 0.625 mg/day (n=120); conjugated estrogens 0.625 mg/day only (n=199); or placebo (n=119). The subjects in all three treatment groups were primarily Caucasian women (87% or more of each group). The results for the incidence of endometrial hyperoplasia in women receiving up to 3 years of treatment are shown below. A comparison of the Prometrium Capsules plus conjugated estrogens treatment group to the conjugated estrogens only group showed a significantly lower rate of hyperplasia (6% combination product versus 64% estrogen alone) in the Prometrium capsules plus conjugated estrogens treatment group throughout 36 months of treatment.

TABLE 3. Incidence of Endometrial Hyperplasia in Women Receiving 3 Years of Treatment

Endometrial Diagnosis	Treatment Group					
	Conjugated Estrogens 0.625 mg + PROMETRIUM Capsules 200 mg (cyclical)		Conjugated Estrogens 0.625 mg (alone)		Placebo	
	Number of patients	% of patients	Number of patients	% of patients	Number of patients	% of patients
	n=117		n=115		n=116	
HYPERPLASIA [a]	7	6	74	64	3	3
Adenocarcinoma	0	0	0	0	1	1
Atypical hyperplasia	1	1	14	12	0	0
Complex hyperplasia	0	0	27	23	1	1
Simple hyperplasia	6	5	33	29	1	1

[a] Most advanced result to least advanced result:
Adenocarcinoma > atypical hyperplasia > complex hyperplasia > simple hyperplasia

In a single-center, randomized, double-blind clinical study that included premenopausal women with secondary amenorrhea for at least 90 days, administration of 10 days of Prometrium Capsules therapy resulted in 80% of women experiencing withdrawal bleeding within 7 days of the last dose of Prometrium Capsules, 300 mg/d (n=20), compared to 10% of women experiencing withdrawal bleeding in the placebo troup (n=21). The rate of secretory transformation was evaluated in a multicenter, randomized, double-blind clinical study in estrogen-primed postmenopasusal women. Prometrium Capsules administered orally for 10 days at 400 mg/d (n=22) induced complete secretory changes in the endometrium in 45% of women compared to 0% in the placebo group (n=23).

10.2 Prior Clinical Research Experience

Sofuoglu and colleagues (2001) previously studied the direct effects of oral progesterone treatment on smoking behavior in female smokers. In each experimental session, following overnight abstinence from smoking, subjects received a single 200 mg dose of progesterone or placebo, orally. Two and a half hours after the medication treatment, subjects were assessed for subjective response to two puffs of a cigarette and then started the self-administration period, in which every 15 minutes, they had the option to exchange their earned token for two puffs of a cigarette or money. Progesterone treatment attenuated subjective rating of good effects of smoking the first cigarette and craving for cigarettes following overnight abstinence. The first cigarette following abstinence is regarded to be highly rewarding and linked to relapse in smokers trying to quit (Brandon et al., 1990; Kenford et al., 1994). The progesterone effects on the subjective rewarding effects of smoking and craving for cigarettes suggested that this hormone may have significant effects on smoking behavior and tobacco withdrawal symptoms. More recently Sofuoglu et al. (in press) studied the effects of two doses of progesterone 200 mg/d and 400 mg/d versus placebo on measures of attention (Stroop and Digit Symbol Substitution Test) and smoking urges in 35 female and 34 male smokers. The 200 mg/d dose improved cognitive performance, while the 400 mg/d dose reduces smoking urges. All subject tolerated both dosages without serious adverse events. There were no effects of progesterone administration on mood, and only modest effects on blood pressure and heart rate, reducing heart rate in males and diastolic blood pressure in females.

The same group conducted a similar preliminary study to examine the progesterone effects on smoked cocaine responses in which cocaine-dependent women, following progesterone or placebo treatment, received three deliveries of 0.4 mg/kg smoked cocaine, 30 min apart. Progesterone treatment attenuated some of the subjective effects of cocaine further suggesting that progesterone may effect mediation of reward by psychostimulants (Sofuoglu et al., 2002a). Preclinical studies provide possible mechanisms to explain these findings. Progesterone has wide-ranging effects in the brain including the activation of GABA type A receptors (Majewska, 1990). Progesterone also affects the dopaminergic system, a neurotransmitter system, which is implicated to be an important neural substrate for both the reinforcing effects of stimulants including cocaine and nicotine and withdrawal symptoms from stimulants (Watkins et al., 2000). Preclinical studies suggest that estradiol and progesterone may have opposing effects on the dopaminergic system. While estradiol has stimulatory effects on the dopaminergic system, progesterone may have inhibitory effects (Becker and Cha, 1989; Dluzen and Ramirez, 1987; Fernandez-Ruiz et al., 1990; Michanek and Meyerson, 1982; Morissette and Di Paolo, 1993; Peris et al., 1991; Roberts et al., 1989; Shimizu and Bray, 1993). These preclinical studies suggest that progesterone may potentially modulate the actions of nicotine through its effects on the dopaminergic system.

Interestingly, there is a growing interest in the neuroprotective effects of progesterone administration for the treatment of traumatic brain injury in both men and women. In a randomized, placebo controlled trial, Wright and colleagues (2007) gave both males and females presenting with acute brain injury a loading dose of progesterone 0.71 mg/kg intravenously followed by approximately 0.5 mg/kg per hour for 11 hours. For the following three days, subjects received progesterone 0.5 mg/kg per hour or placebo for 12 hours each day. At this rate, an average man of 170 pounds (77 kg), would have received a 54.7 mg of progesterone over the first hour followed by 424 mg over the next 11 hours. These doses were well tolerated by both male and female patients, with no serious adverse events attributable to progesterone administration. In fact, 30-day mortality rate was significantly less in those subjects randomized to progesterone compared to those randomized to placebo. This smaller study has led to a large NIH-funded Phase III clinical trial of progesterone administration for traumatic brain injury.

While Prometrium was not the type of progestin used in the Women's Health Initiative Study (WHI), the outcome of this study has led to revision of the Prometrium package insert. Please refer to the insert (enclosed with this IND application) for full details.

10.3 Clinical Care Experience

Physicians world-wide prescribe progesterone to women with an intact uterus who are using estrogen as hormone therapy. Those clinicians who are interested in using oral natural progesterone would prescribe Prometrium as it is the only FDA approved oral micronized progesterone. Our clinical research program has used Prometrium for a number of years. Women presenting for our research studies focusing on estrogen will be prescribed Prometrium 200 mg/d for 12 days in order to obtain a withdrawal bleed after use of unopposed estrogen for 8-12 weeks. We have had no clinically significant adverse events in the over 30 women who have participated in our menopause studies to date.

11 Additional Information

11.1 *Drug dependence and abuse potential – not applicable to this study.*

11.2 Radioactive drugs – not applicable to this study.

11.3 Pediatric studies – not applicable to this study.

11.4 Other information

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