Effect of Modulating CYP3A4 Activity on Mineral Homeostasis

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A. PURPOSE OF RESEARCH

Vitamin D is an important regulator of intestinal CYP3A4 (the enzyme responsible for metabolizing a majority of orally administered drugs) and the intestinal calcium transport proteins. Evidence suggests that CYP3A4 activity influences calcium homeostasis by catabolism of the main circulating vitamin D species, 25-hydroxy vitamin D_3 and its active metabolite, 1α,25-dihydroxy vitamin D_3. The purpose of this study is to test that hypothesis by evaluating the effect of compounds known to induce (rifampin) or inhibit hepatic (clarithromycin) and/or intestinal (clarithromycin, grapefruit juice) CYP3A4 catalytic activity on biomarkers of calcium homeostasis in healthy adults. This study is not designed to assess the safety or efficacy of the comparator treatments. No conclusions should be drawn in that regard. It is a mechanistically informative clinical study.

B. RESEARCH PROCEDURES INVOLVED

Study Design:

This is an open label, randomized, 3 period protocol to be conducted in healthy adults (18-60 yrs). A targeted enrollment of 120 participants pre-screened for good health will be assigned to one of six comparator groups by randomization. There will be 20 participants in each group. The three sequential study periods will be a 7-day Baseline phase, a 14-day Comparator phase and a 14-day Washout phase. During the Comparator phase of the study, participants will take one of the following by mouth for 14 days based on the group to which they were assigned: 1) 200 mL of water; 2) rifampin 600 mg; 3) 200 mL of grapefruit juice; 4) 200 mL of grapefruit juice plus rifampin 600 mg; 5) clarithromycin 250 mg twice a day and 6) clarithromycin 250 mg twice a day plus rifampin 600 mg once a day.

Study Procedures:

Screening. After providing informed consent, participants will be scheduled for a screening medical history and physical examination at the University of Washington General Clinical Research Center. The participants will also undergo an electrocardiogram (EKG) and screening blood test (metabolic panel). A urine pregnancy test will be performed on all women of child-bearing age. Screening procedures will take approximately one hour. If participants qualify for the study, they will start the baseline period of the study within 2 weeks of the screening visit.

Demographic and Inclusion and Exclusion Criteria.

a. *Age*. This is not a treatment study. Its purpose is to determine the effect of changes in CYP3A4 activity on vitamin D homeostasis in healthy adults. Children under 18 years of age or adults older than 60 years of age will be excluded.

b. *Gender.* We aim to recruit an equal number of males and females. However, rifampin and clarithromycin should not be used in pregnant women due to the risk for birth defects. Pregnant and lactating women will be excluded from this study. Women of childbearing age will be required to utilize a barrier method of birth control during the study and for 30 days after completion of the study.

c. *Ethnic and racial minority study composition.* We aim to recruit ethnic and racial minority populations and achieve representation of minorities congruent with the population of King County, Washington.

d. *Inclusion criteria.* 1) Participants will not have a history of significant cardiac, pulmonary, gastrointestinal or renal disease; 2) They will not have a history of HIV, diabetes or Hepatitis B or C; 3) Participants will be males or females between the ages or 18 and 60 years of age; 4) Participants must be able to understand and read English; 5) Participants will be able to provide

informed consent; 6) Subjects must be willing and able to avoid prescription medications, OTC drugs, dietary supplements and foods that are known to modulate CYP3A4 expression or activity; 7) Women will not be currently pregnant or lactating. In addition women participants of childbearing age must be willing to utilize a barrier method of birth control (sterilization will be acceptable); 8) Participants will not have allergies to rifampin, clarithromycin or grapefruit juice; 9) Subject's corrected QTc interval obtained by electrocardiogram will be ≤ 430 ms in men or ≤ 450 ms in women

e. *Exclusion criteria.* 1) People less than 18 years or older than 60 years of age; 2) People with significant cardiac, pulmonary or renal disease, people with diabetes, HIV or Hepatitis B or C; 3) People unable to read and understand English; 4) People unable to provide informed consent; 5) People unwilling or unable to avoid prescription medications, over the counter drugs, dietary supplements and food that are known to alter CYP3A4 expression or activity; people not willing to maintain the recommended diet of consistent calcium and Vitamin D levels during the study or not willing to maintain a daily diary of their food consumption; 6) Women who are pregnant or lactating and women participants of childbearing age not willing to utilize a barrier method of birth control (sterilization will be acceptable); 7) People with allergies to rifampin, clarithromycin or grapefruit juice, people with a corrected QTc interval of > 430 ms in men or > 450 ms in women on electrocardiogram.

General study procedures. This will be a 35-day study, divided into three periods: Baseline, d1-d7; Comparator, d8-d21; Washout, d22-35. The participants will be asked to eat a diet that maintains a consistent amount of vitamin D and calcium for the entire 35 days of the study and will record their daily food intake in a digital diary. They will be given a list of medications and foods to avoid during the study, because they are known to modify CYP3A4 activity.

Baseline Period. Participants will return to the University of Washington Laboratory Services on days 1, 4 and 7 between 8 am - 12 noon for a blood draw. On day 1, they will receive a specimen container for 24-hour urine collection. Urine collection will start on day 6 and continue for 24 hours. Participants will return this completed urine specimen when they return to University of Washington Medical Center on day 7 for blood collection.

Comparator Period. On day 8, the participants will enter the comparator period of the study. Depending on which of 6 groups group to which they were randomized, the participants will take either (1) 200 mL of water at 8 am for 14 days; (2) rifampin 600 mg with 200 mL of water at 8 pm for 14 days; (3) 200 mL of grapefruit juice at 8 am for 14 days; (4) 200 mL of grapefruit juice at 8 am plus rifampin 600 mg at 8 pm, both for 14 days; 5) clarithromycin 250 mg twice a day at 8 am and 8 pm for 14 days and 6) clarithromycin 250 mg twice a day at 8 am plus rifampin 600 mg with 200 mL of water at 8 pm, both for 14 days.

The grapefruit juice utilized in this study will be obtained from the Florida State Department of Citrus because they have a history of providing a high quality product that has been standardized for CYP3A4 inhibition for studies such as this one. Rifampin and clarithromycin will be dispensed by prescription from the University of Washington Investigation Drug Services.

Participants will return to University of Washington Laboratory Services on days 8, 9,12,15,18 and 21 of the study for a blood draw between 8 am - 12 noon. On day 8, the participants will receive a specimen container for the second 24 hour urine collection. Participants will begin this urine collection on day 20 and return this specimen on day 21 when they return to University of Washington Medical Center. The 24 hour urine collection will need to be refrigerated during the collection period.

Washout Period. During the washout period of 14 days the participants will maintain a consistent diet of Vitamin D and calcium intake. On days 23, 25, 28, 31 and 35, the participants will return to University of Washington Laboratory Services for a blood draw between 8 am - 12

noon. On day 23, the participants will receive a specimen container for the second 24 hour urine collection. Participants will begin this urine collection on day 34 and return this specimen on day 35 when they return to University of Washington Medical Center. The 24 hour urine collection will need to be refrigerated during the collection period. The study will conclude with the return of the 24-hr urine specimen and the d35 blood draw.

C. ANALYTICAL METHODS

The primary study outcome measures – serum PTH and osteocalcin, plasma calcium concentration and 24-hr urinary calcium and phosphate concentration (normalized for creatinine concentration) – will be measured by the UWMC Department of Laboratory Medicine using established clinical procedures. The concentration of vitamin D_3 species in plasma will be measured in our research lab HPLC-Mass Spectrometry, using published methods developed by our research team.

D. STATISTICAL ANALYSIS PLAN

We will summarize demographic characteristics by treatment group using mean (SD) for continuous variables and frequency (percent) for categorical variables. We define the baseline value of each analyte to be the average of the three pre-randomization measurements, and the post-treatment value to be the analyte measurement after 14 days of treatment. We will use a linear mixed model with random intercepts and main effects for treatment group, the indicator of time (pre- vs. post-treatment), and the interaction of the two. From this model, we will extract the pre-treatment and post-treatment means, the change in analyte comparing pre- vs post-treatment for each group, and the difference in change in analyte comparing each active treatment group with the change observed in the water group. We will use a Wald test to test each difference in change compared with water, and a global Wald test to test the null hypothesis that all pre- vs post-treatment changes were the same across treatment group.