Dr. Mary F. Paine, RPh, PhD
Washington State University

Title: Evaluating the pharmacokinetics and drug interaction potential of the botanical dietary supplement cinnamon

NCT: 05157672

Document date: 11/15/2024

Dr. Mary F. Paine, RPh, PhD
Washington State University

CLINICAL STUDY PROTOCOL SYNOPSIS

Evaluating the pharmacokinetics and drug interaction potential of the botanical dietary supplement cinnamon

PURPOSE

Cinnamon is used worldwide as both an additive and a botanical dietary supplement, the latter of which ranked within the 25 top-selling herbal supplements in 2019.[1] Cinnamon is added to a variety of products, ranging from foods (*e.g.*, breakfast cereals, baked goods) to fragrances and essential oils, to improve taste or smell. As a dietary supplement, cinnamon is commonly used to lower blood sugar and reduce inflammation.[2] Cinnamon contains the abundant component, cinnamaldehyde, a phenylpropanoid that emanates the flavor and scent of cinnamon. Harrelson and colleagues[3,4] have shown cinnamaldehyde to inhibit the drug metabolizing enzyme cytochrome P450 (CYP) 2A6 in a time-dependent manner. That is, CYP2A6 metabolizes cinnamaldehyde to a reactive intermediate that destroys the enzyme. Such substrates are also referred to as "suicide substrates". This type of enzyme inhibition is similar to that of grapefruit juice, which contains furanocoumarins that are time-dependent inhibitors of CYP3A in the intestine, leading to numerous potential adverse interactions with drugs metabolized by CYP3A. Unlike competitive inhibitors, time-dependent inhibitors inactivate the enzyme permanently, requiring *de novo* synthesis of the enzyme. As such, drug interactions with time-dependent inhibitors can last for several days.

Relative to CYP3A, the list of clinically relevant CYP2A6 substrates is very short. However, two critical substrates include nicotine and the anticancer agent letrozole. Using an *in vitro-to-in vivo* extrapolation approach, cinnamaldehyde was predicted to increase the area under the plasma concentration *vs*. time curve (AUC) of both substrates by 4- to 5-fold,[4] exceeding the FDA recommended cutoff (1.25).[5] These compelling observations prompted our Center of Excellence for Natural Product Interaction Research (NaPDI Center) (U54 AT008909; Paine PI) to conduct a basic experimental study in humans (BESH). The mission of the NaPDI Center is to provide leadership and guidance on how best to determine the clinical relevance of pharmacokinetic interactions between natural products and conventional medications.[6]

The **objective** of this proposed BESH is to evaluate a well-characterized, commercially available cinnamon dietary supplement as a precipitant of pharmacokinetic interactions with CYP2A6 substrates in healthy volunteers. Nicotine gum will be used as the CYP2A6 probe drug (*i.e.*, positive control) and letrozole as a high-impact object drug. Results will be used to inform future research on the potential use of cinnamon as a smoking cessation agent, as well as the clinical impact on pharmacotherapeutic regimens involving letrozole in cancer patients.

The proposed BESH will not evaluate the ability of the selected cinnamon product to diagnose, cure, mitigate, treat, or prevent a disease.

STUDY DESIGN

Healthy non-smoking adult volunteers will be recruited to participate in this BESH. An overview of the study sequence is provided. Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria (see *Page 3*) will be enrolled.

Subjects will participate in Arm 1 and/or Arms 2-5 as follows:

- Arm 1: Administer a single oral dose of a well-characterized, commercially available cinnamon product (2 g) to 6 subjects (3 males, 3 females); they may or may not elect to participate in Arms 2-5. If a subject chooses to participate in Arms 2-5, a washout of at least 7 days will occur between the Arm 1 administration of cinnamon and the Arm 2 administration of nicotine. Blood will be drawn at -0.5, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, and 48 hours. Urine will be collected into multiple jugs from 0-24 hours.
- Arm 2: Administer a single dose of nicotine gum (2 mg) to 16 subjects (8 males, 8 females). Blood will be drawn at -0.5, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after nicotine administration. Urine will be collected into multiple jugs from 0-12 hours.
- Arm 3: Following a washout of at least 24 hours, administer a single oral dose of letrozole (2.5 mg) to the same 16 subjects. Blood will be drawn at -0.5, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 96, 144, 192, and 240 hours after letrozole administration. Urine will be collected into multiple jugs from 0-24 hours.

- Arm 4: Following a washout of at least 14 days, the same 16 subjects will self-administer the cinnamon product (2 g) three times daily for five consecutive days, monitored via a secure video link (e.g., Zoom, FaceTime). On the sixth day, subjects will be administered cinnamon (2 g) and nicotine gum (2 mg). Cinnamon (2 g) will be administered two additional times during this inpatient visit. Blood will be drawn at -0.5, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after cinnamon/nicotine administration. Urine will be collected into multiple jugs from 0-12 hours.
- Arm 5: Following a washout of at least 24 hours, the same 16 subjects will self-administer the cinnamon product (2 g) three times daily for five consecutive days, monitored via a secure video link (e.g., Zoom, FaceTime). On the sixth day, subjects will be administered cinnamon (2 g) and letrozole (2.5 mg). Cinnamon (2 g) will be administered two additional times during this inpatient visit. Blood will be drawn at -0.5, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 96, 144, 192, and 240 hours after cinnamon/letrozole administration. Urine will be collected into multiple jugs from 0-24 hours.

Study procedures. The study will consist of five arms. Arm 1 will include one 14-hour inpatient visit, followed by two outpatient visits. Arms 2 and 4 will each include one 14-hour inpatient visit, followed by six outpatient visits. Arms 3 and 5 will each include one 14-hour inpatient visit. Arms 4 and 5 will additionally include five consecutive days involving self-administration of cinnamon prior to the inpatient visits. If a subject who participates in Arm 1 elects to participate in Arms 2-5, they will undergo a washout period of at least 7 days after Arm 1 cinnamon administration and before participating in Arm 2. During Arm 1, subjects will receive a single dose of cinnamon (2 g) by mouth as a capsule. The cinnamon product to be administered will be supplied by our longstanding natural products chemistry collaborators affiliated with the NaPDI Center, Drs. Nicholas Oberlies and Nadja Cech at the University of North Carolina at Greensboro. In addition to sourcing and chemically characterizing the product (Ceylon variety), they will ensure the product is safe for human consumption by testing for adulterants, microbes, and other contaminants. Finally, they will ensure the product is low in coumarin content such that the total daily dose is below the tolerable daily intake (0.1 mg/kg/day). During Arm 2, subjects will receive a single oral dose of nicotine gum (2 mg). During Arm 3, subjects will receive a single oral dose of letrozole (2.5 mg). During Arm 4, subjects will self-administer cinnamon (2 g) three times daily for five consecutive days by mouth as a capsule. On the sixth day of Arm 4, they will be administered cinnamon (2 g) and nicotine gum (2 mg); they will be administered two additional doses of cinnamon (2 g) during this inpatient visit. During Arm 5, subjects will self-administer cinnamon (2 g) three times daily for five consecutive days by mouth as a capsule. On the sixth day of Arm 5, they will be administered cinnamon (2 g) and letrozole (2.5 mg); they will be administered two additional doses of cinnamon (2 g) during this inpatient visit.

During each inpatient visit, blood (5 mL) will be collected 30 minutes before and at designated times after administration of the cinnamon product and/or the study drugs. There are currently no human pharmacokinetic studies involving cinnamon, but a study in rats reported a half-life of cinnamaldehyde after oral administration of 500 mg/kg to be approximately 7 hours.[7] Thus, blood will be collected from 0-48 hours during Arm 1, or approximately 5 half-lives, to fully characterize the pharmacokinetics of cinnamaldehyde and potentially other cinnamon constituents. The half-life of nicotine in humans is approximately 2 hours,[8] whereas that of letrozole is approximately 2 days.[9] Thus, blood will be collected over 0-11 days during Arms 3 and 5, or approximately 5 half-lives of letrozole, to fully characterize the pharmacokinetics of each analyte.

Blood samples will be immediately processed or will be stored for up to 30 minutes on ice and then processed. Plasma will be harvested from blood cells *via* centrifugation and will be divided evenly among three 1.5-mL Eppendorf screw cap tubes labeled only with subject ID number as the identifier. The Eppendorf tubes will be placed in freezer boxes, which will be stored in a -80°C freezer.

INCLUSION/EXCLUSION CRITERIA

Potential eligible subjects, based on the inclusion/exclusion criteria, will present for a screening visit at the Clinical Research Unit in the Nursing Building on the Washington State University Health Sciences campus in Spokane. A member of the study team will consult with the participants and address any questions or concerns. Informed consent and the HIPAA authorization form will be obtained after full explanation of the study protocol. Consented subjects will undergo the following:

- A medical history and physical exam
- CBC with differential, electrolytes, liver function tests, serum creatinine
- Routine urine analysis

- Urine pregnancy test for female subjects of child-bearing potential (prior to enrollment in the study and prior to each phase of the study using in-home urinalysis pregnancy screens)

Inclusion:

- Males and females, aged from 18-65 years and healthy
- Not taking any medications (prescription and non-prescription) or dietary/herbal supplements known to alter the pharmacokinetics of either study drug or cinnamon constituents
- Willing to abstain from consuming dietary/herbal supplements and citrus juices for several weeks
- Willing to abstain from consuming caffeinated beverages or other caffeine-containing products the evening before and morning of the first day of a study arm
- Willing to abstain from consuming any alcoholic beverages for one day prior to any study day, during the 14-hour inpatient days, and for the 6 outpatient visit(s) following the 14-hour visit
- Willing to use an acceptable method of contraception that does not include oral contraceptive pills or patches (such as abstinence, copper IUD, condom)
- Have the time to participate
- Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for the subject to comply with the requirements of the study

Exclusion:

- Males and females under the age of 18 or over the age of 65 years
- Any current major illness or chronic illness such as (but not limited to) kidney disease, hepatic disease, diabetes mellitus, hypertension, coronary artery disease, chronic obstructive pulmonary disease, cancer, or HIV/AIDS
- History of anemia or any other significant hematologic disorder
- History of drug or alcohol addiction or major psychiatric illness
- Female and pregnant or nursing
- History of allergy to cinnamon, letrozole, or nicotine
- Taking concomitant medications, both prescription and non-prescription (including dietary supplements/herbal products), known to alter the pharmacokinetics of either study drug or cinnamon constituents
- Presence of a condition or abnormality that, in the opinion of the investigator, would compromise the safety of the patient or the quality of the data
- Recreational drug use such as amphetamines, benzodiazepines, cocaine, cannabis, MDMA, opioids, and PCP
- History of intolerance to cinnamon
- Out-of-range clinical laboratory value that the study physician considers participation in the study a health risk

Cinnamon product acquisition. A well-characterized, unadulterated, contaminant-free cinnamon product has been acquired by our natural products chemist collaborators at the University of North Carolina at Greensboro, specifically Drs. Nadja Cech and Nicholas Oberlies. The Cech/Oberlies team leads the Analytical Core of the NaPDI Center and is responsible for sourcing, acquiring, and characterizing our natural products of interest – using grant funds only – to be administered to human participants. Neither this team nor other co-investigators of the NaPDI Center have any financial interest in the manufacture or marketing of the cinnamon product to be used, and the manufacturer is neither providing this product free of charge nor at a discounted rate. The specific product selected, *Cinnamomum verum* (https://ceyloncinnamonshop.com/products/organic-ceylon-cinnamon-supplements-capsules), contains <0.1 mg/g. Considering the maximum total dose to be administered during Arm 3 (6 g), the total daily coumarin intake equates to <0.6 mg, which is well below the tolerable daily intake for an adult (0.1 mg/kg/day).[10]

Nicotine gum and letrozole acquisition. As with our previous clinical studies involving drug products, Dr. John White, our longstanding collaborator/co-investigator and registered pharmacist in Washington state, will oversee the acquisition and storage of oral pharmaceutical grade study drugs.

Pharmacokinetic and statistical analysis. Blood and urine samples will be collected for analysis of letrozole, nicotine, nicotine metabolites, and cinnamon constituents. The pharmacokinetics of each analyte will be determined using traditional noncompartmental analysis methods and the pharmacokinetics analysis software, Phoenix WinNonlin (v8.3, Certara, Princeton, NJ). A two one-sided testing procedure will be used for the primary endpoint analysis as recommended by the FDA for drug-drug interactions studies.[5] Specifically, if the treatment/baseline ratio of log-transformed letrozole

AUC lay between the predefined no-effect range (0.80-1.25), a cinnamon-letrozole interaction will not be evident. Secondary endpoints (*e.g.*, treatment/baseline ratio of C_{max}, $t_{1/2}$, t_{max}, and oral clearance for letrozole and treatment/baseline ratio of AUC, C_{max}, $t_{1/2}$, t_{max}, and oral clearance for nicotine and metabolites will be evaluated using a paired Student's *t*-test or Wilcoxon signed-rank test as appropriate; a p-value <0.05 will be considered statistically significant. For Arm 1, six evaluable subjects, who may or may not elect to participate in Arm 2, are deemed sufficient for a robust pharmacokinetic characterization of cinnamon constituents. For Arm 2-5, a sample size of 16 evaluable subjects will provide 80% power to detect a 20% change in the primary endpoint (letrozole AUC) with a Type I error of 0.05, assuming 26% intra-individual variability in letrozole AUC.[11]

ROLES OF STUDY PERSONNEL

Mary Paine, RPh, PhD
- Principal investigator
- Oversee all aspects of the study

Deena Hadi, BS
- Program manager
- Recruit eligible participants
- Coordinate study days with staff and participants
- Manage finances and study supply logistics

Matthew Layton, MD, PhD
- Physician of record/co-investigator
- Obtain medical history and perform physical exam
- On-call physician for the duration of clinical activities

John White, PA-C, PharmD
- Authorized licensed designee of Dr. Layton/co-investigator
- Obtain medical history and perform physical exam
- Acquire study drugs

Judy Griffin, RN
- Attending registered nurse
- Administer study medications and ensure participant safety
- Place indwelling venous catheters and perform blood collections

James Nguyen, PharmD
- Study coordinator/co-investigator
- Recruit eligible participants
- Conduct screening and clinical activities
- Process and analyze biofluids

REFERENCES

1. Smith T, May G, Eckl V, Morton R (2020) US Herbal Supplement Sales Increase by 8.6% in 2019, Record-Breaking Sales Predicted for 2020. *HerbalGram* 127:54-69.
2. Leach MJ, and Kumar S (2012) Cinnamon for Diabetes Mellitus. *Cochrane Database of Systematic Reviews*.
3. Chan J, Oshiro T, Thomas S, Higa A, Black S, Todorovic A, Elbarbry F, Harrelson JP (2016) Inactivation of CYP2A6 by the Dietary Phenylpropanoid *trans*-Cinnamic Aldehyde (Cinnamaldehyde) and Estimation of Interactions with Nicotine and Letrozole. Drug Metab Dispos 44:534-43.
4. Espiritu MJ, Chen J, Yadav J, Larkin M, Pelletier RD, Chan JM, GC JB, Natesan S, Harrelson JP (2020) Mechanisms of Herb-Drug Interactions Involving Cinnamon and CYP2A6: Focus on Time-Dependent Inhibition by Cinnamaldehyde and 2-Methoxycinnamaldehyde. *Drug Metab Dispos* 48:1028-43.
5. U.S. Food and Drug Administration. Drug Interactions: Relevant Regulatory Guidance and Policy Documents: www.fda.gov/drugs/drug-interactions-labeling/drug-interactions-relevant-regulatory-guidance-and-policy-documents (accessed May 20, 2020).
6. Paine MF, Shen DD, McCune JS (2018) Recommended Approaches for Pharmacokinetic Natural Product-Drug Interaction Research: a NaPDI Center Commentary. *Drug Metab Dispos* 46:1041-5.

7. Zhao H, Xie Y, Yang Q, Cao Y, Tu H, Cao W, Wang S (2014) Pharmacokinetic study of cinnamaldehyde in rats by GC–MS after oral and intravenous administration. *J Pharm Biomed Anal* 89:150-7.
8. Benowitz NL, Jacob III P (1993) Nicotine and Cotinine Elimination Pharmacokinetics in Smokers and Nonsmokers. *Clin Pharmacol Ther* 53:316-23.
9. Jin S-J, Jung JA, Cho S-H, Kim U-J, Choe S, Ghim J-L, Noh O-H, Park H-J, Kim J-C, Jung J-A, Lim H-S, Bae K-S (2012) The Pharmacokinetics of Letrozole and its Association with Key Body Mass Metrics. *Int J Clin Pharmacol Ther* 50:557-65.
10. Abraham K, Wöhrlin F, Lindtner O, Heinemeyer G, Lampen A (2010) Toxicology and Risk Assessment of Coumarin: Focus on Human Data. Mol Nutr Food Res 54, 228-39.
11. Filipe A, Almeida S, Spínola AC, Trabelsi F, Ortuño J (2008) Bioequivalence Study of Two Letrozole Tablet Formulations. Single Dose, Randomized, Open-Label, Two-Way Crossover Bioequivalence Study of Letrozole 2.5 mg Tablets in Healthy Volunteers Under Fasting Conditions. *Arzneimittelforschung* 58:419-22.