Published in final edited form as:

J Clin Pharmacol. 2016 July ; 56(7): 875–884. doi:10.1002/jcph.673.

Impact of *UGT2B17* Gene Deletion on the Pharmacokinetics of 17-hydroexemestane in Healthy Volunteers

Shanly M. Chen, PharmD¹, Daniel H. Atchley, PhD², Michael A. Murphy, MD³, Bill J. Gurley, PhD⁴, and Landry K. Kamdem, PharmD, PhD¹

¹Harding University College of Pharmacy, Arkansas, USA

²University of Pikeville, Kentucky College of Osteopathic Medicine, Kentucky, USA

³Harding University Physician Assistant Program, Arkansas, USA

⁴University of Arkansas for Medical Sciences, College of Pharmacy, Arkansas, USA

Abstract

Exemestane is an aromatase inhibitor drug used for the treatment of hormone-dependent breast cancer. 17-hydroexemestane, the major and biologically active metabolite of exemestane in humans is eliminated via glucuronidation by the polymorphic *UGT2B17* phase II drug metabolizing enzyme. Previous microsomal studies have shown that *UGT2B17* gene deletion affects the intrinsic hepatic clearances of 17-hydroexemestane *in vitro*. In this open-label study, we set out to assess the effect of *UGT2B17* gene deletion on the pharmacokinetics of 17-hydroexemestane in healthy female volunteers with and without *UGT2B17*. To achieve this goal, 14 healthy postmenopausal women (eight carriers of the homozygous *UGT2B17* wild-type allele and six carriers of the homozygous *UGT2B17* gene deletion allele) were enrolled and invited to receive a single 25 mg oral dose of exemestane. Pharmacokinetics was assessed over 72 hours post-dosing. Our results showed that there were statistically significant differences in plasma 17-hydroexemestane AUC_{0-∞} ($p = 0.0007$) and urine 17-hydroexemestane C_{24hr} ($p = 0.001$) between *UGT2B17* genotype groups. Our data suggest that *UGT2B17* gene deletion influences 17-hydroexemestane pharmacokinetics in humans.

Keywords

17-hydroexemestane; exemestane; *UGT2B17* gene deletion; pharmacokinetics and pharmacogenetics; breast cancer

INTRODUCTION

Exemestane is a drug used for the treatment of hormone-receptor positive breast cancers. It works by covalently and irreversibly inhibiting aromatase, the rate limiting enzyme

Corresponding author: Landry K. Kamdem, Pharm.D., Ph.D., Harding University College of Pharmacy, Department of Pharmaceutical Sciences, 915 E. Market Avenue, Searcy, AR 72149-2230, Box 12230, Phone: 501-279-4837, Fax: 501-279-5202 lkamdem@harding.edu.

Disclosure: The authors have no conflict of interest to disclose.

responsible for the peripheral conversion of androgens (testosterone and androstenedione) into estrogens (estradiol and estrone) in postmenopausal women¹⁻³. The major metabolite of exemestane in humans is 17-hydroexemestane. Preclinical studies have shown that 17-hydroexemestane is biologically active⁴⁻⁸. It has bone-protective activities in rats and is a potent aromatase inhibitor *in vitro*⁴⁻⁸.

17-hydroexemestane is a substrate of *UGT2B17* and is cleared via glucuronidation by this polymorphic phase II drug metabolizing enzyme^{9,10}. A recent microsomal study demonstrated that *UGT2B17* gene deletion affects the intrinsic hepatic clearance of 17-hydroexemestane *in vitro*^{9,10}. In their study, Sun et al. found that 1) the rate of 17-hydroexemestane-glucuronide formation was shown to be significantly ($P < 0.001$) decreased (14-fold) in human liver microsomes (HLMs) exhibiting the *UGT2B17*(*2/*2) deletion genotype versus wild-type *UGT2B17*(*1/*1) HLMs; 2) a 36-fold lower intrinsic clearance ($V_{\max}/K_m$) ($P = 0.023$) was observed in *UGT2B17*(*2/*2) versus *UGT2B17*(*1/*1) HLMs; and 3) a significant ($P < 0.0001$, $r^2 = 0.72$) correlation was observed between HLM-mediated 17-hydroexemestane glucuronide formation and liver *UGT2B17* mRNA expression^{9,10}.

Given the high frequency of the *UGT2B17* gene deletion (~30% prevalence in Caucasians and African-Americans)¹¹⁻¹³ and its potential significant importance in the metabolism of 17-hydroexemestane, this study was designed to assess the impact of *UGT2B17* gene deletion on 17-hydroexemestane pharmacokinetics in healthy postmenopausal female volunteers.

METHODS

Study subjects

Study subjects were enrolled with written informed consent under a protocol that was reviewed and approved by the Institutional Review Board at Harding University. All participants were healthy, postmenopausal female volunteers judged to be in good medical condition based on history, physical exam, and blood and urine chemistry results. Postmenopausal status was confirmed by an FSH level between 23-116 mU. Inclusion criteria included: 1) age greater than 45 years at the time of enrollment, 2) postmenopausal as defined by absence of menstruation for 12 months or 6 months if subject had bilateral oophorectomy, 3) healthy with no medical conditions requiring the use of prescription medications, and 4) normal liver function tests (LFTs) (AST, ALT ≤ 75 U/L) as well as normal values for hemoglobin (12-15 gm/dL), platelet count ($150-400 \times 10^3/\mu\text{L}$), and thyroid-stimulating hormone (0.4 – 4.2 $\mu\text{U/mL}$). Participants were ineligible for participation with any of the following: 1) hypersensitivity to exemestane or related compounds, 2) use of prescription medications, 3) history of alcohol or drug abuse, 4) blood donation within the last 2 months, 5) BMI of less than 18.5 Kg/m² or more than 35 Kg/m², 6) inability to give informed consent 7) enrollment in a study within the past 2 months, and 8) smoking.

Study design

This was a nonrandomized, open-label study with subjects selected based on their *UGT2B17* genotype. This study was designed and powered on the basis of a previous preclinical study¹⁰. To detect a statistically significant 2-fold change between *UGT2B17**1/*1 and *UGT2B17**2/*2 genotyping groups in the pharmacokinetic parameters of 17-hydroexemestane with a power of 85% (α -level 5%), 14 participants (eight with the *UGT2B17**1/*1 genotype and six with the *UGT2B17**2/*2 genotype) were found to be sufficient and were enrolled in this investigation. In preparation for the study, subjects were asked to abstain from the use of alcoholic beverages, garlic, citrus products (especially grapefruit and grapefruit juice) as well as apple and grape foodstuffs for at least one week prior to the study. Volunteers were required to stop all over-the-counter medications, caffeinated beverages, herbal or dietary supplements 2 days prior to drug administration. All subjects were pre-screened no more than 6 weeks before drug administration. Pre-screening was comprised of a complete history and physical examination, and laboratory studies including a complete blood count, comprehensive metabolic panel, urinalysis, and *UGT2B17* genotyping. On study day 1, baseline blood was collected via upper extremity peripheral venipuncture and a urine sample was collected prior to dosing. After a breakfast consisting of approximately 30% fat, 45% carbohydrates, 25% protein, and 300 ml orange juice, subjects were then dosed with exemestane 25 mg within 15-30 min. Blood samples were taken via intravenous catheterization at 0.5, 1, 2, 4, 6, and 8 hr after drug administration on study day 1. Catheters were removed and subjects were allowed to go home. Subjects returned to provide urine and blood samples for pharmacokinetic and pharmacodynamic analyses on study days 2 and 4. Subjects were required to return within 24 hours ($\pm$ 2 hours) of the initial dosing time of the study drug on study day 1. Ten milliliters of blood were collected at each visit and used for quantitation of exemestane and 17-hydroexemestane concentrations. Urine samples were also used to assess exemestane and 17-hydroexemestane concentrations. All blood samples were collected in green top (sodium heparin) vacutainer blood collection tubes. Collected samples in tubes were placed on ice immediately after collection and centrifuged at high speed for 10 min within 15 min of collection. Plasma samples were removed and stored at -80°C until analysis. For each study participant, the total amount of blood drawn during the entire study was less than 70 ml. An exit examination, performed at the end of the study was comprised of a complete history and physical examination as well as safety laboratory studies including full blood count, urea, electrolytes, liver function tests (bilirubin, alkaline phosphatase, transaminases, albumin, and total protein), serum lipids (cholesterol and triglycerides), and serum glucose.

UGT2B17 genotyping

Genomic DNA was extracted from whole blood using a Promega's Maxwell biorobot (Madison, WI, USA). DNA quantity and purity was determined photometrically at 260 nm and 280 nm using the Thermo Scientific Nanodrop 2000 C spectrophotometer (Wilmington, DE, USA) according to the manufacturer's instructions. *UGT2B17* deletion genotyping was carried out as described previously by Gallagher et al. using real-time polymerase chain reaction (RT-PCR) with allelic discrimination¹⁴. Each reaction well included two primers and one FAM-labeled probe to amplify exon 1 of *UGT2B17*, and two primers and one 6-JOE-labeled probe (spanning the deletion cut site) that amplify only in the presence of

UGT2B17 deletion. The primer and probe sequences for the *UGT2B17* genotyping assay were as follows: exon 1 (forward) 5'-TGAAAATGTTTCGATAGATGGACATATAAGTA-3', exon 1 (reverse) 5'-GACATCAAATTTTGACTCTTGTAGTTTTC-3', exon 1 (probe) 5'-FAMTACATTTTGGTCATATTTTTCACAACTACAAGAATTGT-3' BHQ1, deletion (forward) 5'-TTTAATGTTTTCTGCCTTATGCCAC-3', deletion (reverse) 5'-AGCCTATGCAATTTTCATTCAACATAG-3', and deletion (probe) 5'-6-JOEACTACACTGAGATTTACAAAAGAATTCTGTCAGGATATAG-3' BHQ1. All primers and probes were obtained from GenScript (Piscataway, NJ, USA). Positive control samples for *UGT2B17**1/*1 (NA11993), *UGT2B17**1/*2 (NA10861), and *UGT2B17**2/*2 (NA12057) genotypes were provided by Coriell. Allelic discrimination was accomplished by RT-PCR amplification performed with the Roche LightCycler 480 detection system (Indianapolis, IN, USA) with the following conditions: 50°C for 2 min, then 95°C for 15 min, followed by 50 cycles of amplification at 95°C for 1 min and 60°C for 90 sec. Data were analyzed with the LightCycler 480 software version 1.5 (Indianapolis, IN, USA).

Determination of exemestane and 17-hydroexemestane concentrations

Previously described LC-MS/MS methodologies were used to quantify exemestane and 17-hydroexemestane in plasma and urine samples of our healthy postmenopausal female volunteers^{9,15}. In order to assess the potential role of glucuronidation in the metabolism of exemestane and 17-hydroexemestane, plasma and urine samples were assayed with and without beta-glucuronidase treatment to reflect total (unconjugated + conjugated) and free (unconjugated) concentrations, respectively. To a 100 µl (200 µl) of subject plasma (urine), 100 µl (200 µl) of 200 units/ml beta-glucuronidase in 75 mM phosphate buffer (pH 7.4) was added. In the absence of beta-glucuronidase, 75 mM phosphate buffer alone was added to plasma or urine samples. The mixture was then allowed to incubate at 37°C for 24 hr. Thereafter, 100 µl (200 µl) of 300 ng/ml internal standard (norgestrel) and 100 µl (200 µl) of acetonitrile were added, vortex-mixed and centrifuged at 6000 g for 10 min in a Sorvall ST 16R centrifuge (Osterode, Germany). The supernatant was then removed and injected onto an LC-MS/MS instrument consisting of an Agilent 1100 HPLC pump (Tokyo, Japan), a CTC Analytics HTCPAC autosampler (Zwingen, Switzerland), and an API 4000 QTRAP MS/MS system (Toronto, Canada). The separation system included a Kinetex 2.6 µm 150 × 4.6 mm column (Phenomenex, Torrance, CA, USA), a security 4 × 3 mm guard column, and a mobile phase consisting of 50% acetonitrile and 50% water at a flow rate of 500 µl/min. The mass spectrometer was operated using electrospray ionization with an ion spray voltage of 5000 V and the temperature was set at 700°C. The positive ion multiple reaction monitoring (MRM) mode was used for analysis and was performed using nitrogen as the collision gas. The nebulizer gas (GS1) and the turbo heater gas (GS2) were set at 20V and 50V, respectively. Declustering potential was 50V for both exemestane and 17-hydroexemestane. A dwell time of 200 ms and a pause time of 5 ms between scans were used to monitor precursor/product ion pairs. Two positive transitions (a quantifier MRM and a qualifier MRM) were used for each analyte. Plasma and urine concentrations of exemestane and 17-hydroexemestane were quantified using the ratio of peak area of exemestane and 17-hydroexemestane to that of the internal standard and calibration curves that were constructed by spiking blank plasma and urine with known amounts of exemestane

and 17-hydroexemestane. Data collection and processing was performed using the Analyst software package (version 1.6, AB SCIEX, Ontario, Canada).

Plasma and urine concentrations of exemestane and its major active metabolite 17-hydroexemestane were quantitated using a sensitive and validated LC-MS/MS method^{9,15}. An API 4000 QTRAP instrument was used for quantitation using the positive electrospray ionization technique and multiple reaction monitoring mode. The monitored precursor to product-ion transitions for exemestane, 17-hydroexemestane, and Norgestrel (the internal standard) were 297->121, 299->135, and 313->109, respectively. Peak areas for exemestane and 17-hydroexemestane were linear from 0 to 200 ng/ml ($r^2 = 0.998$). The lower limit of quantitation for exemestane and 17-hydroexemestane were 0.2 and 0.1 ng/ml, respectively. The between-run imprecision (n = 10) was 6.45%. The total chromatographic run time was 13 min. The retention times of 17-hydroexemestane, exemestane, and internal standard were 7.5, 9.5, and 10.7 min, respectively. A representative chromatogram of exemestane, 17-hydroexemestane, and internal standard is shown in **Fig. 1**

Chemicals

17-hydroexemestane was obtained from Toronto Research Chemicals Inc. (Toronto, Canada). Exemestane, norgestrel (internal standard), beta-glucuronidase, and phosphate buffer were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of HPLC grade or of the highest grade commercially available.

Data analysis

Pharmacokinetic and statistical analyses were performed using Microsoft Excel (version 2013) and Prism (version 6, Graph Pad software, San Diego, CA), respectively. C_{max} and T_{max} were obtained by inspection. AUC_{0-8} was calculated by the linear trapezoidal method for the observed values. $AUC_{0-\infty}$ was determined by extrapolation to infinity. The demographics (BMI, age, and weight) and the pharmacokinetic (C_{max} , T_{max} , AUC_{0-8} , and $AUC_{0-\infty}$) were compared with the subjects' genotype using a two-sided unpaired nonparametric Mann-Whitney U Test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Subject characteristics and *UGT2B17* genotyping data

14 healthy, postmenopausal female volunteers were selected to participate in an open-label nonrandomized exemestane pharmacokinetic clinical study. The subject characteristics of these healthy female volunteers are described in **Table 1**. Eight women carried the *UGT2B17*1/*1* genotype and the remaining six were homozygous for the *UGT2B17*2* allele. There were no statistically significant differences in body mass index, age, and weight between genotype groups (**Table 1**). Thirteen participants completed the study without adverse events. One subject complained of severe headaches prior to the start of the study due to caffeine withdrawal. The subject's symptoms were resolved within the next 24 hours. All participants completed the study.

UGT2B17 genotyping was carried out using a previously described fluorescent-based assay^{14,16}. A representative plot of *UGT2B17* genotyping is shown in **Supplemental Figure F1**.

Impact of *UGT2B17* Gene Deletion on Exemestane pharmacokinetics

The area under the plasma concentration-time curve of exemestane (EXE AUC₀₋₈) and 17-hydroexemestane (17-HEXE AUC₀₋₈) were calculated based on plasma concentrations at predose and at 0.5, 1, 2, 4, 6, and 8 hours after exemestane administration in the presence and absence of beta-glucuronidase using the linear trapezoidal rule. Plasma concentrations at 24 and 72 hours after exemestane administration were not calculated because they were below the lowest limit of quantification. **Fig. 2** shows the plasma concentration time profiles of exemestane and 17-hydroexemestane of 14 healthy postmenopausal female volunteers who received a single oral 25 mg dose of exemestane. 24 hr urine concentrations of exemestane (EXE C_{24hr}) and 17-hydroexemestane (17-HEXE C_{24hr}) were determined using LC-MS/MS as described in the METHODS section. The impact of *UGT2B17* gene deletion on exemestane pharmacokinetics was assessed using a two-sided unpaired nonparametric Mann-Whitney U Test.

In the absence of beta-glucuronidase (–BG), there were no significant differences in the pharmacokinetics (AUC and C_{max}) of exemestane and 17-hydroexemestane between genotype groups (**Tables 2A & 3A and Fig. 2C & 2D**).

In the presence of beta-glucuronidase (+BG), EXE AUC₀₋₈, EXE AUC_{0-∞}, and urine EXE C_{24hr} did not differ significantly between *UGT2B17**1/*1 and *UGT2B17**2/*2 carriers (EXE AUC₀₋₈ 19.8 ± 5.5 vs 19.0 ± 0.5 ng × hr/ml, *p* = 0.5; EXE AUC₀₋₈ 16.2 ± 3.5 vs 15.5 ± 4.2 ng × hr/ml, *p* = 0.7; EXE C_{24hr} 1.7 ± 1.0 vs 2.1 ± 1.6 ng/ml, *p* = 0.8) (**Tables 2B & 3B and Fig. 2A**). However there were statistically significant differences in 17-HEXE AUC_{0-∞}, 17-HEXE AUC₀₋₈ and 17-HEXE C_{24hr} between genotype groups (17-HEXE AUC_{0-∞}, 11.7 ± 7.1 vs 1.6 ± 0.7 ng × hr/ml, *p* = 0.0007; 17-HEXE AUC₀₋₈ 7.3 ± 2.6 vs 0.9 ± 0.2 ng × hr/ml, *p* = 0.0007; 17-HEXE C_{24hr} 2.3 ± 1.2 vs 0.5 ± 0.3 ng/ml, *p* = 0.001) (**Tables 2B & 3B and Fig. 2B**).

The distribution of pharmacokinetic parameters of exemestane and 17-hydroexemestane based on *UGT2B17* genotype in the presence and absence of beta-glucuronidase is shown in **Fig. 3A-D** and **Fig. 4A-D** for C_{max} and AUC₀₋₈, respectively. We also assessed the influence of *UGT2B17* gene deletion on parent drug to metabolite ratio. Our analysis showed that exemestane to 17-hydroexemestane AUC_{0-∞} ratio differed significantly and statistically between *UGT2B17**1/*1 and *2/*2 carriers in the presence of beta-glucuronidase (**Fig. 5B**, *p* = 0.0007).

DISCUSSION

This pilot, clinical pharmacogenetic study reports for the first time an association between *UGT2B17* gene deletion and exemestane metabolism in humans *in vivo*. In our study, we enrolled healthy volunteers that either expressed (*n* = 8 *1/*1) or did not express (*n* = 6 *2/*2) *UGT2B17*. Plasma and urine samples were assayed with and without beta-

glucuronidase treatment to reflect total (unconjugated + conjugated) and free (unconjugated) concentrations, respectively. Our results showed that there were statistically significant differences in the total plasma 17-hydroexemestane $AUC_{0-\infty}$ ($p = 0.0007$), AUC_{0-8} ($p = 0.0007$) and urine 17-hydroexemestane C_{24hr} ($p = 0.001$) between *UGT2B17* genotype groups. These findings suggest that *UGT2B17* gene deletion influences 17-hydroexemestane pharmacokinetics in humans.

Several phase I exemestane single dose pharmacokinetic clinical studies in postmenopausal volunteers have been conducted in the past. Most of these studies were designed to assess the efficacy and safety of exemestane following a single oral 25 mg dose. Others were carried out to study the effects of hepatic and renal impairment, or of concomitant raloxifene administration on the pharmacokinetics of exemestane¹⁷⁻¹⁹. In these studies, the pharmacokinetic parameters of exemestane and its major metabolite 17-hydroexemestane were calculated. They found that plasma concentrations over time (AUC, ng × hr/ml) and maximal plasma concentrations (C_{max} , ng/ml) of exemestane and 17-hydroexemestane ranged from 25.6 to 98.1 ng × hr/ml and 2.46 to 20.89 ng × hr/ml, and from 3.0 to 16.1 ng/ml and 0.22 to 2.44 ng/ml, respectively. In our pharmacokinetic study, AUC and C_{max} of exemestane and 17-hydroexemestane ranged from 9.2 to 40.6 ng × hr/ml and 0.7 to 26.9 ng × hr/ml, and from 2.2 to 13.3 ng/ml and 0.1 to 2.7 ng/ml, respectively. This indicates that our pharmacokinetic data concur with previously reported studies¹⁷⁻¹⁹.

In 2010, Sun et al. observed a significant correlation between 17-hydroexemestane glucuronide formation and *UGT2B17* expression *in vitro*¹⁰. Using a panel of 110 human liver microsomes with different levels of *UGT2B17* mRNA (64 high expressers or **1/*1*; 34 intermediate expressers or **1/*2*; and 12 poor expressers or **2/*2*), Sun et al. showed that there was a 36-fold difference in the intrinsic clearance of 17-hydroexemestane via UGT-mediated deactivation between high and poor expressers¹⁰. We validated this *in vitro* finding in our human pharmacogenetic study *in vivo*, as we observed an almost 8-fold difference in the AUC and C_{max} of conjugated 17-hydroexemestane between high and poor expressers.

Previous research studies have demonstrated a relationship between *UGT2B17* gene deletion and human physiology and pathophysiology. *UGT2B17* is known to be involved in the metabolism of endogenous estrogens and androgens, such as estradiol and testosterone^{20,21}. Juul et al. showed that *UGT2B17* strongly affects urinary excretion patterns of androgen metabolites in pubertal boys. They observed that subjects with a homozygous deletion of *UGT2B17* had significantly lower urinary levels of testosterone and 5 α - and 5 β -androstanediol. A similar trend was seen in a larger case-control genome-wide copy number variation study, which aimed at identifying the genetic markers associated with the pathogenesis of osteoporosis²¹. In their study involving subjects consisting of 689 Chinese and 1000 Caucasians, Yang et al. demonstrated that individuals with zero copies of *UGT2B17* (poor expressers) had significantly higher serum testosterone and estradiol concentrations. They also showed that Caucasian and Chinese individuals, with either one or two copies of *UGT2B17*, had lower bone mineral density and increased risk of osteoporosis. These studies suggest that *UGT2B17* may play a significant role in human growth and disease. In our current study, we report for the first time a link between *UGT2B17* gene deletion and exemestane disposition *in vivo*.

What are the clinical implications of our findings? It is generally accepted that aromatase inhibitor drugs exemestane, anastrozole, and letrozole are clinically equivalent. Consequently, oncologists have used these drugs interchangeably with the assumption that they exhibit similar risk-to-benefit ratios^{3,22-24}. This may not be appropriate because it has been observed in clinical practice that patients who complain of serious adverse events, such as arthralgia, with one aromatase inhibitor can tolerate another. This suggests that there are potentially significant differences between aromatase inhibitors and that there is significant interindividual variability in response to this pharmacological class of agents²⁵⁻²⁸. Some studies have attempted to address this by demonstrating that distinct pharmacological differences exist between steroidal (exemestane) and nonsteroidal aromatase inhibitors (letrozole and anastrozole)^{4-6,29-32}. Preclinical studies in ovariectomized rats have shown that exemestane is less toxic to bone than nonsteroidal aromatase inhibitors⁴⁻⁶. In these preclinical studies, 17-hydroexemestane was identified as the primary molecule responsible for exemestane-induced bone protection. Some clinical studies have validated these animal findings by demonstrating that women receiving non-steroidal aromatase inhibitors have significantly lower bone mineral densities than those treated with exemestane²⁹⁻³². However, several other clinical studies have not replicated these observations. We hypothesize that the failure to demonstrate superiority or inferiority of exemestane over nonsteroidal aromatase inhibitors in larger clinical trials may be due to significant inter-individual variability in the formation of 17-hydroexemestane. In this pilot study, we showed that the interindividual variability in 17-hydroexemestane metabolism and disposition is dependent on *UGT2B17* genotype status. Given the high frequency of the *UGT2B17* gene deletion (*UGT2B17**1/*1: 48-53% versus *UGT2B17**1/*2: 39-40% and *UGT2B17**2/*2: 8-12%)¹¹⁻¹³, future pharmacogenetic studies aimed at assessing the differences in exemestane pharmacokinetics and pharmacodynamics as well as breast cancer outcomes among these three *UGT2B17* genotyping groups are now warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This publication was made possible by the 2011 Young Investigator Award supported by grant funding from the American Society for Clinical Pharmacology and Therapeutics (ASCPT) and by the Arkansas INBRE program, supported by grant funding from the National Institute of Health (NIH) National Institute of General Medical Sciences (NIGMS) (P20 GM103429). We thank Mr. David Crouch for helping with subject recruitment. We are very thankful to study volunteers, White County Medical Center, students, faculty and staff of the Harding community for their participation and contribution. We are especially grateful to Drs. Kathryn Momary, Susan Rahman, and Reggie Ewesuedo for the mentorship they exhibited throughout the conduct of this clinical study.

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Fig. 1. Representative Multiple Reaction Monitoring (MRM) chromatogram of exemestane, 17-hydroexemestane, and norgestrel (internal standard) of a spiked plasma sample. The retention times of 17-hydroexemestane, exemestane, and internal standard were 7.5, 9.5, and 10.7 min, respectively.

Fig. 2. Pharmacokinetic of exemestane and 17-hydroexemestane stratified by *UGT2B17* genotype. Concentration vs. time plots of exemestane and 17-hydroexemestane was measured with a validated LC-MS/MS assay in subject plasma samples up to 72 hr after administration of a single oral 25 mg exemestane dose. Plasma samples were analyzed with beta-glucuronidase (+BG) and without incubation with beta-glucuronidase (-BG). A (Exemestane +BG), B (17-hydroexemestane +BG), C (exemestane -BG), and D (17-hydroexemestane -BG). Circles and squares represent *UGT2B17**1/*1 and *UGT2B17**2/*2 genotypes, respectively. The bars depict standard deviations of the mean.

Fig. 3. C_{max} of exemestane and 17-hydroexemestane stratified by *UGT2B17* genotype. Plasma samples were analyzed with beta-glucuronidase (+BG) and without incubation with beta-glucuronidase (-BG). A (Exemestane -BG), B (exemestane +BG), C (17-hydroexemestane -BG), and D (17-hydroexemestane +BG). Circles and squares represent *UGT2B17**1/*1 and *UGT2B17**2/*2 genotypes, respectively.

Fig. 4. AUC_{0-∞} of exemestane and 17-hydroexemestane stratified by *UGT2B17* genotype. Plasma samples were analyzed with beta-glucuronidase (+BG) and without incubation with beta-glucuronidase (-BG). A (Exemestane -BG), B (exemestane +BG), C (17-hydroexemestane -BG), D (17-hydroexemestane +BG). Circles and squares represent *UGT2B17**1/*1 and *UGT2B17**2/*2 genotypes, respectively.

Fig. 5. Ratio of AUC_{0-∞} of exemestane over AUC_{0-∞} of 17-hydroexemestane stratified by *UGT2B17* genotype. Plasma samples were analyzed with beta-glucuronidase (+BG) and without incubation with beta-glucuronidase (-BG). A (Exemestane AUC_{0-∞} / 17-hydroexemestane AUC_{0-∞} -BG) and B (Exemestane AUC_{0-∞} / 17-hydroexemestane AUC_{0-∞} +BG). Circles and squares represent *UGT2B17**1/*1 and *UGT2B17**2/*2 genotypes, respectively.

Table 1

Descriptive data of study participants.

Demographics	<i>UGT2B17</i> Genotype		<i>P</i> values
	*1/*1 (n = 8)	*2/*2 (n =6)	
BMI Kg/m ² (SD)	26.2 (4.9)	27.5 (5.8)	0.9
Age, years (SD)	57.5 (6.2)	54.0 (4.0)	0.4
Weight, Kg (SD)	70.3 (13.6)	74.2 (15.5)	0.9

SD, standard deviation.

Table 2

Pharmacokinetic (PK) of exemestane and 17-hydroexemestane of serum samples processed in the absence (A) and presence (B) of beta-glucuronidase. The data are stratified by *UGT2B17* genotype.

A. Without beta-glucuronidase						
	Exemestane			17-hydroexemestane		
PK Parameter, unit	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values
AUC _{0-∞} , ng × hr/ml (SD)	22.7 (10.5)	19.7 (3.8)	0.7	1.2 (0.8)	1.3 (0.3)	0.4
C _{max} , ng/ml (SD)	5.6 (10.5)	5.3 (1.4)	0.7	0.2 (0.1)	0.2 (0.1)	0.9
T _{max} , hr (SD)	3.1 (1.2)	1.7 (1.2)	0.1	3.6 (1.5)	2 (1.1)	0.06
B. With beta-glucuronidase						
	Exemestane			17-hydroexemestane		
PK Parameter, unit	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values
AUC _{0-∞} , ng × hr/ml (SD)	19.8 (5.5)	19.0 (3.3)	0.5	11.7 (7.1)	1.6 (0.7)	0.0007
C _{max} , ng/ml (SD)	4.4 (1.6)	5.2 (1.5)	0.3	1.7 (1.5)	0.2 (0.1)	0.0007
T _{max} , hr (SD)	3.1 (1.2)	1.9 (1.2)	0.1	3.5 (0.9)	2 (1.1)	0.02

AUC, area under the plasma concentration-time curve; SD, standard deviation;

Table 3

24 hr concentrations of exemestane and 17-hydroexemestane of urine samples processed in the presence and absence of beta-glucuronidase. The results are stratified by *UGT2B17* genotype.

A. Without beta-glucuronidase						
Exemestane			17-hydroexemestane			
PK Parameter, unit	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values
C _{24 hr} , ng/ml (SD)	1.9 (0.9)	2.5 (2.1)	0.7	0.4 (0.2)	0.4 (0.3)	0.9
B. With beta-glucuronidase						
Exemestane			17-hydroexemestane			
PK Parameter, unit	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values	<i>UGT2B17</i> *1/*1 (n = 8)	<i>UGT2B17</i> *2/*2 (n = 6)	<i>P</i> values
C _{24 hr} , ng/ml (SD)	1.7 (1.0)	2.1 (1.6)	0.8	2.3 (1.2)	0.5 (0.3)	0.001

SD, standard deviation; C_{24 hr}, 24-hr urine concentration.