Divergent Effects of Raloxifene HCl on the the Pharmacokinetics and Pharmacodynamics of Warfarin

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Purpose. Evista® (raloxifene HCl) is a nonsteroidal selective estrogen receptor modulator that displays estrogen agonist effects on bone and lipid metabolism but estrogen antagonist effects on the breast and endometrium. The potential for drug-drug interaction between raloxifene and warfarin was assessed in 15 healthy postmenopausal women.

Methods. Single doses of warfarin (20 mg) were administered prior to and during 2 weeks of dosing with raloxifene 120 mg/day. Each warfarin dose was followed by pharmacokinetic sampling and prothrombin time measurements.

Results. Raloxifene administration resulted in 7.1% and 14.1% decreases in the clearance (CL_P/F) and 7.4% and 9.8% decreases in the volume of distribution (V_{SS}/F) of R- and S-warfarin, respectively (all $p \leq 0.05$). In contrast to the slightly higher plasma concentrations of R- and S-warfarin, raloxifene reduced the maximum prothrombin time (PT_{max}) by 10% and the area under the PT versus time curve from 0–120 h (AUC_{PT}) by 8% ($p < 0.01$).

Conclusions. Raloxifene administration may result in a small increase in systemic warfarin exposure that is associated with a diminution, not augmentation, of the pharmacodynamic effect. Due to the small magnitude of this effect, concomitant administration of raloxifene and warfarin is not likely to result in clinically significant drug-drug interaction.

KEY WORDS: raloxifene; SERM; warfarin; drug-drug interaction.

INTRODUCTION

Evista® (raloxifene hydrochloride, Fig. 1) is a tissue-selective estrogen receptor modulator (SERM) that is indicated for the prevention and treatment of postmenopausal osteoporosis. Raloxifene has estrogen agonist effects on bone metabolism and lipid metabolism but not on the breast or endometrium in postmenopausal women. Raloxifene therapy is associated with an increased incidence of venous thromboembolism (VTE) (1) which is similar to that observed with postmenopausal estrogen replacement therapy (2) and another nonsteroidal SERM, tamoxifen (3). In addition to VTE incidence, many women at risk for osteoporosis or breast cancer may have other conditions such as prosthetic heart valves and atrial fibrillation that require prophylactic anticoagulation with warfarin. The clinical safety of SERM or estrogen therapy in women requiring concomitant warfarin therapy has not been assessed to date in adequately controlled clinical trials. Nonetheless, a large number of such women may present to clinicians with a clinical need for the prevention or treatment of osteoporosis.

One determinant of the safety of concomitant use of warfarin and raloxifene is the potential for a drug-drug interaction between these agents. Warfarin is a racemic mixture of R- and S- enantiomers, which are pharmacologically active in inhibiting the regeneration of active vitamin K involved in γ-carboxylation of Factors II, VII, X. In humans, S-warfarin is five times more potent (4) and is eliminated more rapidly than R-warfarin. Hepatic metabolism is the most important route of warfarin elimination in humans (5). The principal metabolism of R- and S-warfarin is catalyzed by a variety of cytochromes P450, resulting in a series of monohydroxylated metabolites that are further metabolized to warfarin alcohols and conjugates. The predominant human CYP isozyme catalyzing S-warfarin metabolism is CYP2C9, whereas CYP1A2 and CYP3A4 are the major contributors to R-warfarin metabolism.

Estrogen agonists may alter warfarin pharmacokinetics by several potential mechanisms including inhibition or induction of CYP enzymes and alterations in protein binding. Raloxifene is metabolized exclusively by glucuronidation, and, hence, is unlikely to affect the metabolism of warfarin. Although raloxifene is highly bound to albumin (~99%) and α1-acid glycoprotein (~89%) *in vitro,* displacement of warfarin from plasma proteins is unlikely since raloxifene circulates at concentrations 20-fold lower than those of warfarin. In addition to pharmacokinetic effects, estrogen agonists may alter warfarin action by pharmacodynamic effects on the concentration or activity of plasma coagulation factors (6). The effect of raloxifene on the activity of vitamin K dependent coagulation factors is unknown.

Drug-drug interaction studies between warfarin and estrogen replacement regimens or other SERMs (e.g. clomiphene, tamoxifen) have not been reported to date. This open label study in healthy postmenopausal women was undertaken to evaluate the effects of multiple doses of raloxifene HCl on the single dose pharmacokinetics and pharmacodynamics of R- and S-warfarin in healthy postmenopausal women.

MATERIALS AND METHODS

Subject Selection

Fifteen healthy postmenopausal female subjects, ages 47 to 72 years, participated in this study. Five of the 15 were cigarette smokers. Fourteen subjects were Caucasian and one was African-American. Prior use of estrogen within 30 days of the study, personal history of VTE, or a first degree relative with a history of VTE were among the exclusion criteria. Subjects were instructed to maintain usual dietary habits during ambulatory periods and avoid large variation in intake of Vitamin K containing foods. Subjects were advised to avoid the use of aspirin and cyclo-oxygenase inhibitors during warfarin dosing periods. The protocol was reviewed by the Institutional Review Committee of Indiana University and Purdue University at Indianapolis. Informed consent was obtained from all study participants prior to enrollment.

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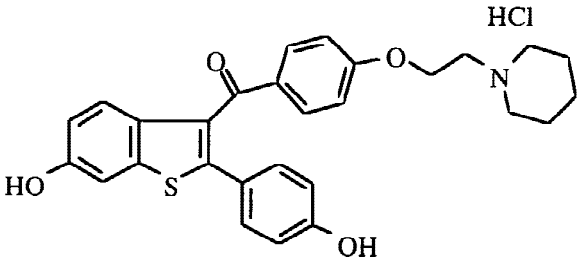

Fig. 1. The chemical structure of raloxifene HCl.

Study Design

The pharmacokinetics and pharmacodynamics of a single 20-mg dose of warfarin were assessed prior to and during a period of steady-state raloxifene dosing. Two 10-mg Coumadin® tablets (DuPont Pharma) were given orally at approximately 7 AM on Days 1 and 16 following an overnight fast of at least 10 h. Plasma samples for assay of R- and S-warfarin and prothrombin time measurements were obtained during a 120-hour period following warfarin dosing.

After completion of the first warfarin sampling period on Day 6, two 60-mg Raloxifene HCl tablets were administered daily for 15 days. After daily administration of raloxifene HCl for a period of approximately eight elimination half-lives (Day 16), a second dose of warfarin (20 mg) was administered and measurements of warfarin pharmacokinetics and pharmacodynamics were repeated. Raloxifene HCl dosing was continued throughout the second warfarin sampling period (Day 20).

Bioanalytical Assay for R- and S-warfarin in Plasma

Samples of venous blood were collected in heparinized tubes (7 mL) prior to and at approximately 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 12, 24, 48, 72, 96, and 120 h after warfarin administration. After collection, plasma was transferred to polypropylene tubes and stored at approximately 20°C until shipped on dry ice to the analytical laboratory (Avtech Laboratories, Kalamazoo, MI). A high-performance liquid chromatography (HPLC) assay with fluorescence detection was used to quantitate the R- and S-enantiomers of warfarin in plasma. The standard curve range was 7.5 to 2500 ng/mL for each enantiomer. During the validation, the relative standard deviation (RSD) was ≤4.4% for both R- and S-warfarin. The absolute mean relative error (RE) was ≤1.5% for both R- and S-warfarin. Quality control samples of plasma spiked with R- and S-warfarin at three different concentrations were analyzed with each set of study samples.

Protein Binding Interaction Study

Protein binding studies were conducted prior to this clinical study, using pooled human plasma from healthy donors. Aliquots of plasma (2 mL, pH 7.4, at 37°C) were incubated for 30 min with ^{3}H-raloxifene hydrochloride (specific activity 31.2 Ci/mmol, New England Nuclear, Boston MA) or ^{14}C-racemic warfarin (specific activity 57.0 mCi/mmol, Amersham International, Buckinghamshire UK). After incubation, plasma aliquots were removed for scintillation counting and for ultracentrifugation at 100,000 RPM for 4 h using a Beckman TL-100 centrifuge set at approximately 37°C. Aliquots of non-centrifuged plasma and centrifuged supernatant were counted in triplicate. Binding interaction studies were performed by incubation with ^{14}C-warfarin for 30 min, followed by addition of ^{3}H-raloxifene for an additional 30 min, and vice versa.

Pharmacokinetic Analyses

Plasma concentration-time data for R-warfarin and S-warfarin were analyzed using noncompartmental pharmacokinetic techniques. Maximum plasma concentration (C_{max}) for warfarin enantiomers and the corresponding sampling time (T_{max}) were recorded as observed. The elimination rate constant (λ_z) was determined as the slope of the linear regression for the terminal log-linear portion of the concentration-time curve. Area under the plasma concentration versus time curve (AUC), area under the first moment curve (AUMC), mean residence time (MRT), oral plasma clearance (CL_P/F), and volume of distribution at steady state (V_{SS}/F) for warfarin enantiomers were calculated according to standard methods. The racemic dose of warfarin contains an equal proportion of R- and S-enantiomers, therefore the total administered dose of warfarin was divided by two when calculating the oral plasma clearance of individual enantiomers.

Pharmacodynamic Analyses

The pharmacodynamic response to each single dose of warfarin was assessed by measurement of the prothrombin time (PT) prior to and at 12, 24, 48, 72, 96, and 120 h after warfarin dosing. For each PT measurement, an initial volume of at least 2.5 mL of blood was drawn into a separate container, then 5 mL of blood was drawn into a tube containing sodium citrate anticoagulant. This sample was inverted several times and placed on ice promptly after phlebotomy. Samples were centrifuged within 30 min of phlebotomy, and plasma was transferred to plastic tubes on ice. Prothrombin time was measured at Indiana University School of Medicine, Indianapolis, IN, using an Electra MLA 1600c photometric clot detection apparatus (Medical Laboratory Automation, Inc, Pleasantville, NY) and Ortho Diagnostics Reagent System® (Johnson & Johnson Co, Raritan, NJ). This reagent system utilizes a recombinant human tissue factor added to purified phospholipids (Recombiplastin®) and has an International Sensitivity Index (ISI) of 1.0. Samples from all subjects at a single time point were analyzed together, and samples from different time points were analyzed at separate times. The tissue factor reagent was the same for all assays, and quality control standards were assayed prior to each assay run. Since the mean normal prothrombin time standard did not change during the study and the international normalized ratio (INR) was linearly related to the PT, only the PT was analyzed as the pharmacodynamic endpoint.

The baseline prothrombin time (PT_b) before the first and the second warfarin dose was recorded for each subject. The maximum prothrombin time values (PT_{max}) were determined by inspection of prothrombin time versus time plots for each subject. The area under the prothrombin time versus time curve from 0 to 120 h (AUC_{PT}) was calculated by the trapezoidal method.

Statistical Analyses

A paired sample *t* test was used to analyze the pharmacokinetic and pharmacodynamic data. Statistical analysis was

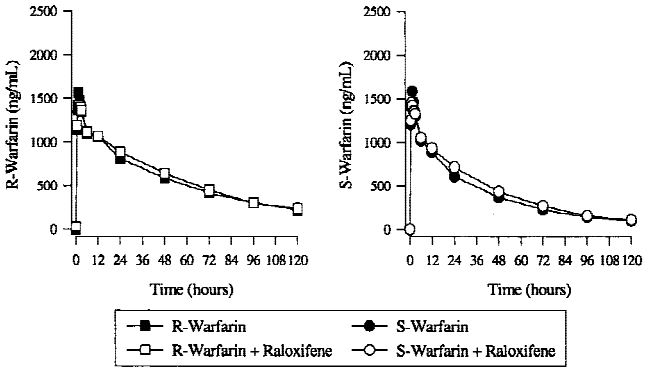

Fig. 2. Plasma concentration vs. time profiles for R-and S-warfarin following administration of 20 mg warfarin with (open symbols) and without (closed symbols) steady-state raloxifene administration (120 mg/day).

performed on several pharmacokinetic parameters for warfarin enantiomers: C_{max}, $AUC_{0-\infty}$, λ_z, CL_P/F, and V_{SS}/F. Due to the non-Gaussian distribution of pharmacokinetic parameters such as AUC and C_{max}, logarithmic transformation was performed prior to statistical testing of the ratio of the geometric means (7). Significance tests were performed at the 5% significance level. T_{max} for warfarin enantiomers was analyzed using a Poisson regression approach. Mean plasma concentrations at each sampling time were determined for graphical evaluation only. Statistical analysis of pharmacodynamic endpoints was performed by paired *t* test on the log-transformed parameters PT_b, PT_{max}, and AUC_{PT}.

RESULTS

Pharmacokinetic Results

The mean plasma concentration vs. time profiles of R- and S-warfarin, alone and in combination with raloxifene, are depicted in Fig. 2. Prior to the second warfarin treatment period, residual concentrations of R- and S-warfarin were detected in 12 and 4 subjects, respectively. The contribution of these residual concentrations to the $AUC_{0-\infty}$ of R- and S-warfarin in the second period was estimated to be 2.0% and 1.6%, respectively. This was considered to be a non-significant carryover effect. One subject ingested naproxen approximately 1 day prior to the second dose of warfarin which resulted in interference with the warfarin assay during the second warfarin treatment period. Warfarin pharmacokinetic data for this subject were excluded from analysis. The results of the pharmacokinetic and statistical analyses are summarized in Tables I and II.

The coadministration of a single dose of warfarin with raloxifene at steady state led to a small, but statistically significant, decrease in the oral clearance (CL_P/F) and the volume of distribution (V_{SS}/F), but not the C_{max} or T_{max} of the warfarin enantiomers. The differences between the mean values for CL_P/F were –7.1% and –14.1% for R- and S-warfarin, respectively. The differences between the mean values for V_{SS}/F were –7.4% and –9.8% for R- and S-warfarin, respectively (Table I). As a result of proportional changes in the clearance and volume of distribution, the half-life of warfarin enantiomers remained unchanged.

Protein Binding Results

When incubated separately, raloxifene (1 ng/mL) was 98.2 ± 0.4% (mean ± SD, $n = 3$) bound to plasma proteins, while racemic warfarin (2.2 μg/mL) was 99.2 ± 0.1% protein bound. Addition of raloxifene to warfarin resulted in warfarin binding of 98.9 ± 0.1%, and raloxifene binding of 98.0 ± 0.5%. Addition of warfarin to raloxifene resulted in warfarin binding of 98.9 ± 0.4% and raloxifene binding of 97.7 ± 1.2%. No clinically significant protein binding displacement was observed.

Pharmacodynamic Results

Mean prothrombin time responses to the 20-mg doses of warfarin are depicted in Fig. 3. The statistical analysis of phar-

Table I. Effect of Raloxifene on the Pharmacokinetic Parameters of R- and S-Warfarin

	Arithmetic Mean (CV as %)			
	R-warfarin		S-warfarin	
Parameter	Warfarin	Warfarin (+Raloxifene)	Warfarin	Warfarin (+Raloxifene)
C_{max}	1682.3	1683.1	1741.9	1716.8
(ng/mL)	(14)	(24)	(13)	(27)
T_{max}	1.0	1.3	1.0	1.0
(hr)	(1.0–2.0)	(0.5–12.0)	(0.5–2.0)	(0.5–3.0)
$AUC_{0-\infty}$	84585.3	89618.6	52892.3	60494.0
(ng · hr/mL)	(26)	(25)	(38)	(37)
CL_P/F	2.1	2.0	3.4	3.0
(mL/min)	(38)	(32)	(35)	(30)
V_{SS}/F	8.3	7.7	9.0	8.1
(L)	(14)	(12)	(14)	(17)
MRT	69.9	68.0	46.7	47.6
(h)	(30)	(24)	(38)	(31)
$t_{1/2}$	45.6	44.7	33.2	32.7
(h)	(24.5–78.1)	(28.5–65 .7)	(23.3–71.0)	(26.1–64.6)

Note. All parameters except T_{max} and $t_{1/2}$ are reported as arithmetic mean (CV%). T_{max} is expressed as median (range) and $t_{1/2}$ as harmonic mean (range).

Table II. Statistical Analysis of Raloxifene Effect on C_{max} and $AUC_{0-\infty}$ of R- and S-warfarin

Parameter	Geometric Mean		Treatment Effect	90% Confidence Interval	*p*-value
	Warfarin	Warfarin (+Raloxifene)			
R-Warfarin					
C_{max} (ng/mL)	1667	1639	0.98[a]	0.91 to 1.06	0.71
$AUC_{0-\infty}$ (ng · h/mL)	81340	86572	1.06[a]	1.03 to 1.10	0.006
λ_z (h^{-1})	0.02	0.02	2.1%[b]	–3.7 to 8.0%	0.53
CL_P/F (L/h)	2.16	2.01	–7.1%[b]	–12.9 to –1.2%	0.05
V_{SS}/F (L)	8.32	7.71	–7.4%[b]	–11.9 to –2.8%	0.01
S-Warfarin					
C_{max} (ng/mL)	1763	1659	0.96[a]	0.86 to 1.06	0.47
$AUC_{0-\infty}$ (ng · h/mL)	49781	57371	1.15[a]	1.09 to 1.21	0.0002
λ_z (h^{-1})	0.02	0.02	1.3%[b]	–4.1 to 6.6%	0.68
CL_P/F (L/h)	3.54	3.04	–14.1%[b]	–21.0 to –7.2%	0.003
V_{SS}/F (L)	9.01	8.13	–9.8%[b]	–14.2 to –5.3%	0.02

[a] C_{max} and AUC treatment eflects are expressed as the ratio of geometric mean parameter values from raloxilene to control period.
[b] λ_z, CL_P/F, and V_{SS}/F treatment effects are expressed as the difference (%) in arithmetic mean parameter values from raloxifene to control period.

macodynamic parameters is presented in Table III. The mean prothrombin time before warfarin dosing was not different from baseline during raloxifene therapy. The maximum prothrombin time after a single 20-mg dose of warfarin was reduced by 10% during raloxifene therapy. The AUC_{PT} of the pharmacodynamic effect was similarly reduced by 8% under these conditions. The effect of raloxifene on the pharmacodynamics of warfarin is not accounted for by changes in warfarin pharmacokinetics, which were in the opposite direction.

DISCUSSION

The key observation of this study is that the small increase in systemic warfarin exposure resulting from raloxifene administration was associated with a diminution, not augmentation, of the pharmacodynamic effect. The pharmacokinetic effect is notable for a reduction in the oral clearance and apparent volume of distribution of both warfarin enantiomers. The pharmacokinetic parameters of the R- and S-warfarin in this study were consistent with literature data (5) and the effect of raloxifene on the clearance and volume of both warfarin enantiomers was similar.

Because the two warfarin enantiomers differ in the pattern of CYP isozymes involved in their metabolism, the observed pharmacokinetic changes may be unrelated to oxidative metabolism. Raloxifene is metabolized exclusively by glucuronidation, not oxidation, hence, inhibition of warfarin metabolism is unlikely. Warfarin is a low hepatic extraction drug for which decreased hepatic metabolism may affect the clearance but is unlikely to alter the apparent volume of distribution. Higher bioavailability due to increased absorption is also unlikely since warfarin is rapidly and extensively absorbed from the gastrointestinal tract (8).

While increased bioavailability and decreased hepatic metabolism of warfarin do not account for the pharmacokinetic results, increased plasma protein binding could account for decreases in clearance and volume of distribution of warfarin enantiomers. Warfarin clearance correlates with its unbound fraction (9). Unbound warfarin constitutes less than 1% of total plasma warfarin, hence, small effects on protein binding may have pharmacokinetic significance. Although raloxifene does not affect plasma albumin concentrations, it may increase the plasma concentrations of other plasma proteins due to its estrogen-like effects (10,11). Warfarin is bound extensively by albumin but also by other plasma proteins (12). The current study provides no direct evidence for this hypothesis, but the observed pharmacokinetic changes are consistent with increased plasma protein binding during raloxifene treatment.

In contrast to the effect of raloxifene on the pharmacokinetics of warfarin, the prothrombin response to warfarin was reduced by chronic raloxifene administration. Increased plasma protein binding which resulted in reduced plasma concentrations of unbound (pharmacologically active) warfarin enantiomers is one possible explanation. However, at least in

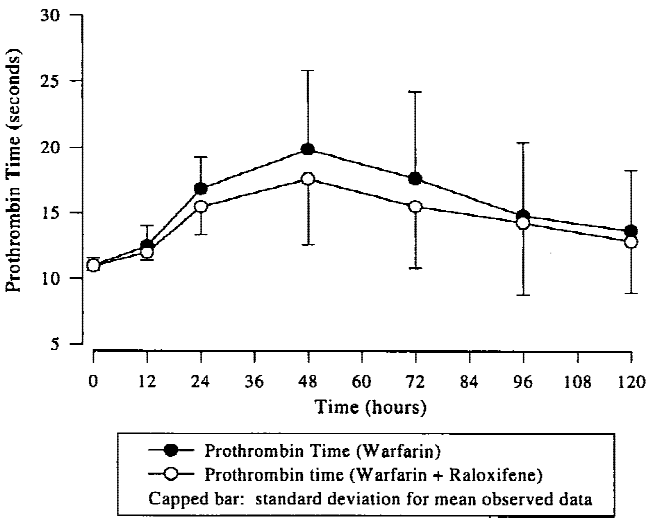

Fig. 3. Prothrombin time vs. time profile following administration of warfarin with (open symbols) and without (closed symbols) steady state raloxifene administration.

Table III. Statistical Analysis of Raloxifene Effect on Warfarin Pharmacodynamics

Parameter	Geometric Mean		Ratio of means	90% Confidence Interval	Significance *p*-value
	Warfarin	Warfarin (+Raloxifene)			
Baseline PT (sec)	10.98	10.94	1.00	0.98 to 1.01	0.73
Maximum PT (sec)	19.6	17.6	0.90	0.87 to 0.93	0.0001
AUC_{PT} (sec · h)	1888	1742	0.92	0.90 to 0.94	0.0001

Note. Interaction with raloxifene is expressed as the ratio of mean parameter values from raloxifene to control period.

theory, this is unlikely due to the fact that unbound plasma concentrations of drugs with low hepatic extraction ratio are not sensitive to changes in free fraction, assuming steady-state conditions (13). Although warfarin was administered as a single dose, the effect on free drug concentrations as a result of increased plasma protein binding should be minimal assuming that the increased plasma protein concentrations were relatively stable during the second warfarin dosing period.

The reduction in prothrombin time response is likely to have been mediated by a pharmacodynamic mechanism such as an estrogen agonist effect of raloxifene on circulating coagulation factors. Several studies have suggested that the plasma concentrations or activity of vitamin K-dependent clotting factors are increased by oral estrogens (14,15,16). One study of the effect of oral contraceptives on the effects of single doses of a coumarin derivative anticoagulant demonstrated that the prothrombin response is blunted by estrogen therapy (6).

Under steady-state raloxifene dosing conditions, the maximal prothrombin time response to a single 20-mg dose of warfarin was reduced by 10%. Warfarin pharmacodynamics show high interindividual variability, and a ten-fold dose range (1 to 10 mg per day) is commonly used in clinical practice. It is likely, therefore, that raloxifene therapy may reduce the prothrombin time of patients on a stable dose of warfarin, but that the magnitude of this effect is likely to be small. The dose of raloxifene used in this study (120 mg/day) was twice the therapeutic dose of raloxifene (60 mg/day). Therefore, the magnitude of raloxifene-warfarin interaction is not likely to be greater under clinical conditions. The manufacturer's recommendation to monitor the prothrombin time after changes in any concomitant medications (17) would likely lead to appropriate dose adjustment. The therapeutic goals of warfarin therapy are not different in pre- and postmenopausal women, suggesting that usual clinical guidelines to warfarin dosing should apply, even in the presence of the estrogenic effects of raloxifene.

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