Dose Proportionality and Population Characteristics of Oral Fadrozole Hydrochloride, an Aromatase Inhibitor, in Postmenopausal Women

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The dose proportionality of the pharmacokinetics of fadrozole was investigated in 18 healthy postmenopausal women. Fadrozole hydrochloride was administered as 0.3-, 1.0-, and 2.0-mg oral doses continuously every 12 hr for 5 days each in a Latin square design. At steady state, the dose-normalized pharmacokinetic parameters AUC and C_{max} were found to be independent of the dose. In addition, no statistically significant differences in t_{max} were detected. It was concluded that the pharmacokinetics of fadrozole were dose proportional in the projected therapeutic dose range. The relationship between oral clearance and the demographic factors, age, weight, and height, was assessed. Oral clearance was related to total body weight but not age or height. Prospective estimates of the population components of variance showed that intersubject variance accounted for 91.7% of the total random variance. Weight variance accounted for 36.1% of the intersubject variance.

KEY WORDS: fadrozole hydrochloride; linear pharmacokinetics; population components of variance.

INTRODUCTION

Fadrozole, 4-(5,6,7,8-tetrahydroimidazo[1,5-a]pyridin-5-yl) benzonitrile monohydrochloride, is a selective inhibitor of estrogen synthesis (1). Fadrozole may have beneficial effects in the treatment of patients with estrogen-dependent breast cancer (2). Preliminary studies indicated that fadrozole is rapidly absorbed after oral administration, with an elimination half-life of about 9 hr (3,15). Less than 5% of the dose is excreted unchanged in the urine, indicating extensive metabolism. In rats and dogs, the predominant pathway of biotransformation is 8-hydroxylation followed by partial glucuronide formation. Although the *in vivo* metabolic transformation of fadrozole in man has not been fully elucidated quantitatively as of yet, 8-hydroxylation followed by glucuronidation appears to be a prominent pathway. This is supported by *in vitro* metabolic studies using human microsomes.[4]

An earlier study in cancer patients gave a preliminary indication that the pharmacokinetics of fadrozole are linear (3). The objective of the current study is to provide statistically rigorous information on the dose proportionality of the pharmacokinetics of orally administered fadrozole hydrochloride. In addition, the population characteristics of fadrozole's oral clearance were prospectively evaluated, and the relationship between oral clearance and demographic factors was assessed. The dosage selected incorporates the currently projected therapeutic range.

MATERIALS AND METHODS

Subjects

Eighteen healthy postmenopausal women with a normal health history, physical examination, chest X-ray, routine laboratory test results, and ECG completed this study. Of the 18 subjects, 39% had experienced natural menopause and 61% had surgically induced menopause. Subjects were excluded from the study if they had taken any form of medication, other than hormone replacement therapy, during the 2 weeks prior to the first drug dose or at any time during the study. All subjects gave written, informed consent before participating in the study. The subjects had a mean age of 54 years (range, 30–72 years). Their mean weight was 65.8 kg (range, 51.8–82.7 kg) and their mean height was 161 cm (range, 152–173 cm). All subjects were Caucasian.

Clinical Study Design and Procedures

This was an open-label, steady-state 3 × 3 Latin square crossover trial. Subjects were randomly assigned to one of six treatment sequences. Each subject received 0.3-, 1.0-, and 2.0-mg doses of fadrozole hydrochloride orally every 12 hr for 5 days each. The order of treatment was randomly assigned. There was no washout interval between the multiple dosing periods.

The morning dose on the fifth day of each treatment period was administered after an overnight fast of at least 10 hr. Food was withheld for a further 4 hr after dosing. No evening dose was administered on the fifth day.

The subjects were carefully observed for signs of drug activity and were questioned about the occurrence of any medical problems during each treatment period and at the termination of the study.

Blood samples were collected prior to administration of any drug and just before the morning and evening doses on the fourth day of each treatment period. On the fifth day of each treatment period, blood samples were serially obtained at 0, 0.5, 1, 2, 4, 6, 8, and 12 hr.

Each blood sample (10 mL) was drawn into an evacuated blood collection tube containing 143 USP U of heparin. The heparinized blood was centrifuged and the separated plasma was transferred to a clean polypropylene tube and frozen. All plasma samples were stored frozen until analyzed for drug concentrations.

Drug Administration

Fadrozole hydrochloride was administered as formulated 0.3- and 1.0-mg immediate-release tablets. The doses were one 0.3-mg tablet, one 1.0-mg tablet, and two 1.0-mg tablets. The doses were administered at approximately 8:00

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AM and 8:00 PM each day during the treatment periods. Throughout the study, tablets were swallowed with about 250 mL of water.

Analytical Methodology

Plasma samples were analyzed for fadrozole by a modified version of a previously reported technique (3). The modifications are as follows. Fadrozole and the internal standard, d_4-fadrozole, were resolved by gas chromatography on a bonded phase dimethylpolysiloxane/silica capillary column (SPB-1, Supelco, Inc., Bellefonte, PA). The column was 15 m in length, with a 0.32-mm i.d. and a 0.25-mm film thickness. A 100-cm guard column was employed (Supelco SPB-5) and changed at the end of each day. The initial oven temperature was 150°C, ramped at a rate of 40°C/min to a final temperature of 245°C. Detection was achieved by selected monitoring of the positive ion chemical ionization mass spectrum. The reactant gas was methane and the molecular ions were monitored at an m/z of 224 for fadrozole and 228 for d_4-fadrozole. Calibration curves were linear in the 0.20–20 ng/mL concentration range and were run daily. Intrassay accuracy and precision (n = 6) was 102% with a coefficient of variation (CV) of 3.5%, 104% with a CV of 3.4%, and 93% with a CV of 8.6% at plasma concentrations of 0.20, 4.0, and 15.0 ng/mL, respectively. The limit of quantitation was 0.20 ng/mL fadrozole. All drug concentrations are reported as that of the free base.

Pharmacokinetic Analysis

Plasma drug concentration–time profiles were characterized in terms of their maximum concentration, C_{max}; time to maximum concentration, t_{max}; and area under the drug concentration–time curve during the 12-hr steady-state dosing interval, AUC(0–12). C_{max} was estimated as the highest observed concentration during the dose interval, and t_{max} was estimated as the corresponding time after dosing. AUC(0–12) was estimated using the linear trapezoidal method. Oral clearance, CL_o, was estimated from the dose, corrected by the molecular weight ratio of free base to salt (0.86), divided by the AUC(0–12).

Statistical Analysis

The pharmacokinetic variables AUC(0–12) and C_{max} were dose normalized by dividing the estimated values for the two higher doses by the ratio of the dose to the smallest dose, 0.3 mg. The dose-normalized values of AUC(0–12) and C_{max} and the raw values of t_{max} were then tested for equivalence to the 0.3-mg dose by the following methods.

An analysis of variance (ANOVA) was performed with treatment sequence, subject within sequence, period, direct dose effects, and carryover dose effects as nominal factors (4). From the resulting direct effect means for direct dose effects, the direct effect means for the two higher doses were compared to the direct effect mean of the 0.3-mg dose. t tests were employed using the ANOVA mean square error. The power was calculated for a 20% difference (α = 0.05) from the direct effect mean of the 0.3-mg dose for each of the two higher doses separately. Each of the relative differences between direct effect means for the higher doses and the 0.3-mg dose was estimated by a conventional 90% confidence interval centered at the relative difference between the direct effects means and a Westlake symmetric 95% confidence interval. The t_{max} values from the two higher doses were compared to the t_{max} for the 0.3-mg dose by the Wilcoxon signed-rank test.

The attainment of steady state was assessed by comparing the fourth- and fifth-day morning predose values for each treatment by a paired t test.

Once it was established that the AUCs were dose proportional, estimates of the population components of variance for CL_o were evaluated. This analysis utilized a mixed-effects model where subject effects were regarded as a random factor and period effects as a fixed factor (5). Sequence and carryover effects were not considered in this model. Accordingly, it was assumed that the CL_o values associated with the different dose levels represented random replicates.

All statistical tests used a 5% level of significance to determine significance. All P values cited for between-dose comparisons were two-tailed.

The relationship between CL_o and the demographic characteristics, age, weight, and height, was evaluated by multiple linear regression. In the event that a significant relationship was obtained, the appropriate population variational component was adjusted for the contributing factors as follows.

$$\sigma^2 = \sigma'^2 + \Sigma\, \omega_i^2 \cdot \sigma_i^2$$

where σ^2 is the component total variance, σ'^2 is the adjusted (partial) variance due to unknown factors, ω_i is the regression coefficient corresponding to known factor i, and σ_i^2 is the variance of factor i.

RESULTS

The mean plasma concentration–time profiles are illustrated in Fig. 1 for each of the 0.3-, 1.0-, and 2.0-mg/12 hr treatments.

Inspection of individual plasma curves revealed that the AUC and C_{max} resulting from the administration of the 0.3-mg dose in one subject were unusually large. Further eval-

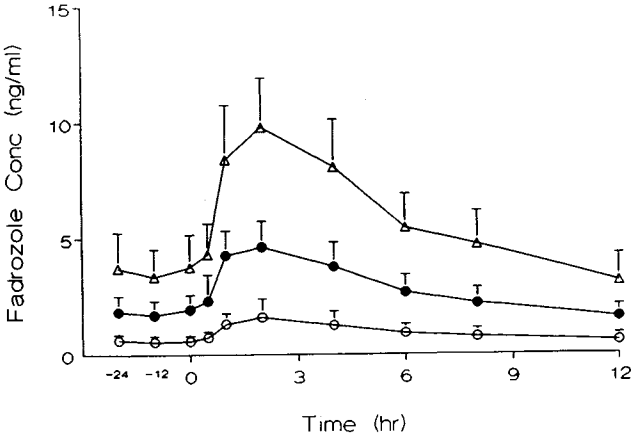

Fig. 1. Steady-state plasma concentrations of fadrozole following the oral administration of 0.3-mg (○), 1.0-mg (●), and 2.0-mg (△) doses of fadrozole hydrochloride every 12 hr for 5 days each. Mean (SD) in 18 healthy postmenopausal women.

uation of plots of residuals versus predicted values from the ANOVAs indicated that this value was a potential outlier. Both the AUC and the C_{max} were two to four times greater than those values for any other subject. Further evaluation showed that a 0.3-mg dose from Period 1 of this trial resulted in unusually low AUC and C_{max} values. It was, therefore, suspected that an inadvertent switch in doses had taken place. Consequently, statistical analysis was performed on the complete set of data and the data set excluding two subjects from Period 1. Since the conclusions were identical, only the results of the excluded data set are presented here.

Attainment of Steady State

The mean (SD) predose plasma levels of fadrozole on the morning and evening on the fourth and fifth days of dosing with the 0.3-mg dose were 0.59 (0.21), 0.52 (0.23), 0.56 (0.20), and 0.50 (0.18) ng/mL, respectively. The corresponding values for the 1.0-mg dose were 1.84 (0.70), 1.69 (0.63), 1.93 (0.65), and 1.62 (0.54) ng/mL, while the values for the 2.0-mg dose were 3.72 (1.54), 3.35 (1.18), 3.75 (1.41), and 3.13 (1.19) ng/mL (Fig. 1).

No significant differences were detected between the morning predose plasma concentration on the fourth day and that on the fifth day of dosing for any treatment, indicating that steady-state conditions had been achieved in each case. Additional comparisons of the morning and evening values on the fourth day and the evening value on the fourth day with the morning value on the fifth day showed the existence of a diurnal effect. In all cases, the morning values were higher than the evening values, with the differences achieving statistical significance for the 1.0- and 2.0-mg doses ($P < 0.01$).

Dose Proportionality

Due to the Latin square design of this trial and the nature of the dosing regimens in which no washout period was employed, a suitable statistical correction was utilized to account for carryover effects. Mean values are therefore reported as direct effect means (Table I). Comparison of raw means and statistically adjusted direct effects means, however, showed that in all cases the correction was of minor consequence. In fact, no statistically significant carryover effects were detected with respect to any parameter in this trial. In addition, no sequence or period effects were detected.

No statistically significant treatment differences were detected for the dose-normalized AUC and C_{max} values or the t_{max} values. Both the power and the confidence intervals greatly exceeded the prescribed minimal criteria of 80 and ±20%, respectively. It can be concluded that the pharmacokinetics of fadrozole are strictly dose proportional in the 0.3 to 2.0-mg dose range in postmenopausal women.

Relationship Between CL_o and Age, Weight, and Height

The relationship between CL_o and the demographic factors was assessed by multiple regression analysis. In this analysis, each subject's average CL_o value was used. The linear factor coefficients (P value) corresponding to age, weight, and height were −2.23 (0.287), −8.03 (0.016), and 2.59 (0.540). None of the factors were pairwise correlated to each other. Since the factors age and height were not significantly related to CL_o, the model was reduced to include only weight. Regression analysis of the reduced model resulted in a coefficient (P value) of −7.78 (0.009) (Fig. 2). The linear correlation coefficient (r) was 0.594, which was significant at $P < 0.01$. These results indicate that the oral clearance of fadrozole was related to the total body weight at steady state in women in this trial.

Table I. Evaluation of Fadrozole Dose Proportionality by ANOVA

	AUC(0–12) (ng · hr/mL)	C_{max} (ng/mL)	t_{max} (hr)[a]
Direct-effect dose-normalized mean (SE)			
0.3 mg	10.7 (0.22)	1.5 (0.04)	2[b]
1.0 mg	10.5 (0.22)	1.5 (0.04)	2
2.0 mg	10.7 (0.21)	1.5 (0.04)	2
P value from between-dose comparison of direct-effect means			
0.3 vs 1.0 mg	0.682	0.372	0.182[c]
0.3 vs 2.0 mg	0.950	0.597	0.586
Power of between-dose comparisons of direct-effect means to detect 20% difference from 0.3 mg			
1.0 mg	>0.99	>0.99	
2.0 mg	>0.99	>0.99	
Conventional 90% confidence interval for relative difference from 0.3-mg direct-effect mean for			
1.0 mg	−3.9%, 6.3%	−10.4%, 3.5%	
2.0 mg	−5.2%, 4.8%	−8.9%, 4.7%	
Westlake 95% confidence interval for relative difference from 0.3-mg direct-effect mean for			
1.0 mg	±6.6%	±10.4%	
2.0 mg	±6.0%	±9.1%	

[a] Unadjusted for dose.
[b] Median.
[c] From Wilcoxon signed-rank test.

Population Characteristics of Oral Clearance

Prospective estimates of the population components of variance showed that the intersubject effect was highly sig-

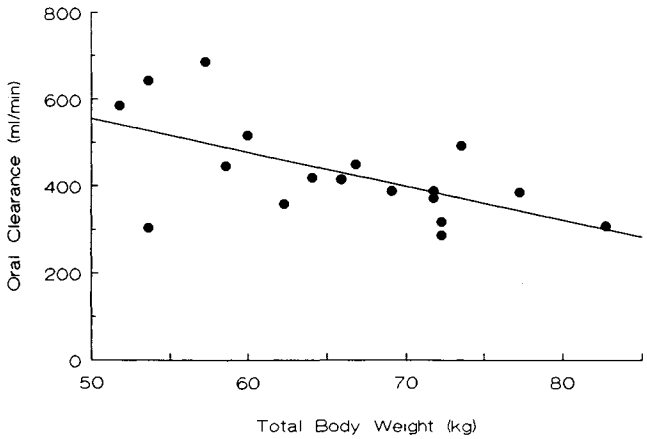

Fig. 2. Correlation of fadrozole's steady-state oral clearance with total body weight. The least-squares line of regression is CL_0 (mL/min) = 943.2 − 7.778 × total body weight (kg); r = 0.594 ($P < 0.01$). This relationship corresponds with postmenopausal women in the 51.8- to 82.7-kg range.

nificant and that it accounted for 91.7% of the total random variance associated with CL_0 (Table II). Since appreciable weight variation during the 15-day duration of this study was considered unlikely, any variational contribution by weight was assumed to correspond to subjects in a unique one-to-one fashion. The intersubject variance was, therefore, considered as the only variational component dependent on weight variability. Partial adjustment of the intersubject variance showed that 36.1% of the unadjusted intersubject variability could be accounted for by the contribution of weight variance. The proportion of random variation contributed by the adjusted intersubject variance was 87.6%. These results indicate that intersubject variance of CL_0 is, by far, the predominant source component of population variability, even after taking into consideration the variational dependence on subject weight.

DISCUSSION

The results of the present study are consistent with the findings of the earlier multiple-dose (every 12 hr) steady-state study in the 4.0- and 8.0-mg dose range (3). The dose range selected in this study reflects that which is currently under investigation in clinical trials and which has been shown to be efficacious with regard to tumor regression (6). The results of the current study establish the linearity of fadrozole's pharmacokinetics.

In animal studies, the recovery of total radioactivity in the urine of rats, dogs, and rabbits following intravenous and oral administration of labeled fadrozole indicated that absorption of oral fadrozole was virtually complete (>94%). These findings suggest that the oral clearance of fadrozole in man is virtually independent of the bioavailable fraction. As such, the observed relationship between oral clearance and body weight may be regarded to represent the relationship between metabolic clearance and body weight. The dependence of metabolic clearance on body weight is not intuitively obvious. Whether body weight is a causative factor or merely an associated factor is not known. It is clear, at least in this study, however, that the steady-state oral clearance declined in linear fashion by up to 44% in the weight range 51.8 to 82.7 kg.

A possible explanation for the observed relationship between fadrozole's metabolic clearance and total body weight may be an intermediate autoregulation of hepatic P-450 oxidation by estrogens. In normal postmenopausal women the major source of estrogen is from the peripheral aromatization of androgens. Since adipose tissue has a relatively high degree of aromatase activity, it might be expected that the rate of estrogen synthesis is related to total body weight in this population. Several investigations have established this dependence (7–9). The effect of estrogens on P-450 oxidation is paradoxical. Estrogens have been shown to inhibit the production of hepatic cytochrome P-450 *in vitro* (10–12). In an isolated organ perfusion study, however, it was shown that a pronounced increase in cyclosporine metabolic rate was associated with administration of ethinyl estradiol (13). The authors of this study concluded that estradiol has a direct effect on hepatic enzyme activity. If fadrozole's metabolism is related in some way to either the synthetic rate of estrogens or the estrogen concentration, one would also expect an association between fadrozole's clearance and dose since fadrozole is an aromatase inhibitor which regulates estrogen concentration. While this was not observed in this study, if the degree of inhibition were near-maximal for any dose selected in a near-maximal dose range, little additional estrogen regulation would be anticipated. Further investigation will be required to evaluate the robustness of this association, causative factors, and sensitivity of the effect to causative factors before any assessment of the role of estrogen on fadrozole's metabolism or any dosing recommendations can be made.

The estimated probabilistic population characteristics of fadrozole's oral clearance corresponded exceedingly well with previously reported characteristics projected from concentration minima at steady state (14). The relative 8.3% contribution from intrasubject variance indicates that the metabolic disposition in individual subjects is stable in the dose range studied. Differences in metabolic capacity between individuals, however, is a major determinant of plasma concentrations and drug disposition. These differ-

Table II. Estimated Population Components of Variance, Steady-State Oral Clearance in Women

CL_0 units	mL/min
F statistic (*P* value)	
For intersubject effect	32.8 (<0.001)
For period effect	0.486 (0.620)
Mean (±SE)	434.3 (±27.29)
Random population variance	
Intrasubject	1,176
Intersubject	12,966
Adjusted intersubject	8,291
Proportion of random variance due to intersubject variability	91.7%
Proportion of adjusted random variance due to adjusted intersubject variability	87.6%
Proportion of intersubject variance due to total body weight variance	36.1%

ences predominate even after removing the contribution of weight variance from the population intersubject variance.

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