0090-9556/97/2505-0598–602$02.00/0
DRUG METABOLISM AND DISPOSITION Vol. 25, No. 5
Copyright © 1997 by The American Society for Pharmacology and Experimental Therapeutics
Printed in U.S.A.

DRUG METABOLISM & DISPOSITION

# INHIBITION OF HUMAN DRUG METABOLIZING CYTOCHROMES P450 BY ANASTROZOLE, A POTENT AND SELECTIVE INHIBITOR OF AROMATASE

SCOTT W. GRIMM AND MARTIN C. DYROFF

*Drug Disposition and Metabolism Department, Zeneca Pharmaceuticals*

(Received October 25, 1996; accepted January 23, 1997)

**ABSTRACT:**

**Anastrozole (2,2′[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]-bis(2-methylproprionitrile)) is a potent third-generation inhibitor of aromatase, currently marketed as a treatment for postmenopausal women with advanced breast cancer. While its potency and selectivity for inhibition of estrogen synthesis has been established in both preclinical and clinical studies, this study used *in vitro* methods to examine the effects of anastrozole on several drug metabolizing CYP enzymes found in human liver. Human liver microsomes were co-incubated with anastrozole and probe substrates for CYP1A2 (phenacetin), CYP2A6 (coumarin), CYP2C9 (tolbutamide), CYP2D6 (dextromethorphan), and CYP3A (nifedipine). The formation of the CYP-specific metabolites following co-incubation with various anastrozole concentrations was determined to establish $IC_{50}$ and $K_i$ values for these enzymes. While anastrozole did not inhibit CYP2A6 and CYP2D6 activities at concentrations below 500 $\mu$M, this compound inhibited CYP1A2, CYP2C9, and CYP3A activities with $K_i$ values of 8, 10, and 10 $\mu$M, respectively. Dixon plots used to determine the $K_i$ values for the inhibition of CYP1A2 and CYP3A activities by anastrozole were biphasic, indicating additional lower affinity $K_i$ values. Major metabolites of anastrozole did not retain the ability to inhibit the metabolism of nifedipine (CYP3A). The results of this study indicate that, although anastrozole can inhibit CYP1A2, 2C9, and 3A-mediated catalytic activities, this compound would not be expected to cause clinically significant interactions with other CYP-metabolized drugs at physiologically relevant concentrations achieved during therapy with Arimidex (Zeneca, Ltd., Macclesfield, UK) 1-mg.**

Downloaded from dmd.aspetjournals.org at ASPET Journals on July 20, 2018

Anastrozole (ZD1033), an achiral triazole derivative known as 2,2′[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methylproprionitrile) (fig. 1), is a potent inhibitor of aromatase (CYP19), which converts androgens to estrogens. This compound is currently marketed as a treatment for postmenopausal women with advanced breast cancer. Anastrozole exhibits high intrinsic potency demonstrated by the *in vitro* inhibition of human placental aromatase with an $IC_{50}$ of 15 nM. Preclinical studies with anastrozole demonstrated its selectivity for aromatase *in vivo* as compared with inhibition of other enzyme activities responsible for steroid biosynthetic pathways. Cholesterol biosynthesis was minimally inhibited *in vitro* and plasma cholesterol concentrations were unchanged in preclinical species at doses that were at least 30 times higher than its maximally effective dose (0.1 mg/kg) required for aromatase inhibition (1). Anastrozole, at doses 100–200 times its maximally effective aromatase inhibitory dose, did not interfere with cholesterol side-chain cleavage nor did it affect plasma aldosterone levels, sodium and potassium excretion, or adrenal and prostate gland weights. Anastrozole was a comparatively weak inhibitor of bovine adrenal 11$\beta$-hydroxylase *in vitro* ($IC_{50} \approx 12$ $\mu$M), and *in vivo* changes in circulating 11-deoxycorticosterone or hypokalemia were not observed at 3 and 10 mg/kg doses in monkeys and dogs, respectively. Androgen synthesis was not affected in rats and monkeys at 10 times or, in dogs, at 100 times the effective aromatase inhibitory dose. The selectivity of anastrozole was also established in early clinical studies where doses of 1 to 10 mg in postmenopausal female volunteers produced maximal suppression of estradiol without affecting cortisol or aldosterone secretion (1, 2).

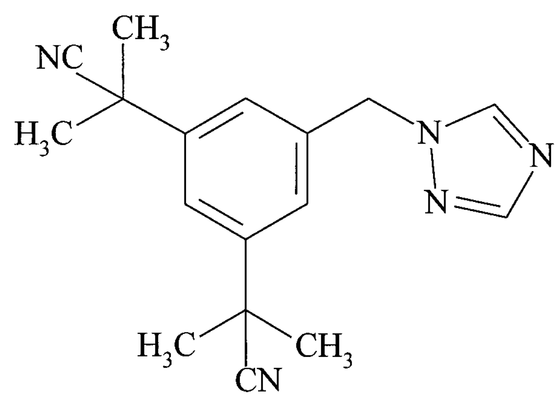


FIG. 1. *Structure of anastrozole.*

Various azole-containing compounds have been shown to be potent inhibitors and inducers of cytochrome P450-mediated drug metabolism both *in vitro* and *in vivo*. Lipophilic compounds containing nitrogen heterocycles such as phenylimidazoles and phenylpyridines inhibit rat liver monooxygenase activities with varying microsomal binding affinities depending on the position of heterocycle substitution (3). Many investigators have demonstrated that *N*- and C4-substituted imidazole drugs such as ketoconazole and cimetidine inhibit CYP[1]activity and

**Send reprint requests to:** S. W. Grimm, LW245, Drug Disposition and Metabolism Department, Zeneca Pharmaceuticals, Zeneca, Inc., 1800 Concord Pike, Wilmington, DE 19850.

[1] Abbreviations used are: CYP, cytochrome P450; BSA, bovine serum albumin; BMPN-benzoic acid, 3,5-bis-(2-methyl-propionitrile)-benzoic acid; PMSF, phenylmethyl-sulfonylfluoride; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TFA, trifluoroacetic acid; TCA, trichloroacetic acid; HCl, hydrochloric acid; MTBE, methyl-tert-butyl ether; $C_{max}$, maximum concentration.

drug clearance in both animals and man (4–8). The potency of inhibition by these agents varies greatly and is determined by their hydrophobic nature and the effect of steric hindrance on the strength of the bond between their heteroatomic lone pair of electrons and the CYP heme iron. Although many azole-containing compounds are generally thought to be nonspecific inhibitors of monooxygenase activity, the selectivity of CYP inhibition by compounds such as ketoconazole has been shown to be concentration-dependent in human *in vitro* systems (9).

Information on potential drug:drug interactions is necessary during the development of all compounds intended for therapeutic use. *In vitro* methodologies are now routinely used to study the potential of a drug candidate to inhibit specific CYP enzymes and classify the drugs most likely to interact during clinical use. This study examined the effects of anastrozole on several of the major drug metabolizing CYP enzymes in human liver using *in vitro* approaches. Comparisons of the inhibitory effects were made to other known inhibitors.

## Materials and Methods

**Chemicals.** Anastrozole, BMPN-benzoic acid, dehydronifedipine, and dextrorphan were synthesized by Zeneca Pharmaceuticals (Macclesfield, UK or Wilmington, DE). Phenacetin and acetaminophen were obtained from US Pharmacopoeia Convention, Inc. (Rockville, MD). Cimetidine, coumarin, dextromethorphan, ketoconazole, nifedipine, tolbutamide, and umbelliferone were obtained from Sigma Chemical Co. (St. Louis, MO). 4-Hydroxytolbutamide was obtained from Ultrafine Chemicals, Ltd. (Manchester, UK). All other reagents and supplies were obtained from standard commercial sources.

**Microsome preparations.** Transplant quality samples of human liver were obtained from the International Institute for the Advancement of Medicine (Exton, PA). Microsomes were prepared from liver tissue immediately upon receipt by differential centrifugation using previously described methods (10). Microsomal suspensions were stored for subsequent use at −80°C in 10 mM Tris-acetate (pH 7.4) containing 0.1 mM EDTA, 0.1 mM PMSF, and 20% glycerol (v/v). The total protein content of each microsomal sample was determined using the bicinchoninic acid reagent (Pierce Chemical Co., Rockford, IL) using BSA as the standard.

**Determination of Human CYP Activities.** Phenacetin deethylation to acetaminophen was used to assess CYP1A2 activity in human liver microsomes. Incubation mixtures of 0.5 ml contained 0.1 mg microsomal protein, 50 mM HEPES (pH 7.5), 15 mM $MgCl_2$, 1 mM NADPH, and phenacetin (50, 100, and 200 $\mu$M). Reactions were stopped after 15 min by the addition of 2 ml isopropanol:ethyl acetate (1:9 v/v). The samples were vortexed and then centrifuged to separate aqueous and organic layers. The organic layer was transferred to a clean tube and evaporated under nitrogen to dryness. Samples were reconstituted in HPLC mobile phase (10:90 acetonitrile: 0.1% TFA in water). The concentration of acetaminophen produced in the microsomal incubation was determined by HPLC with UV detection at 248 nm. Separation of acetaminophen from phenacetin and other reaction components was accomplished using a Zorbax SB-C8 column (MacMod Analytical, Inc., Chadds Ford, PA), 4.6 × 75 mm, with a precolumn 0.5 $\mu$m filter (Upchurch Scientific, Oak Harbor, WA). Acetaminophen (rt = 1.6 min) was eluted from the column using acetonitrile: 0.1% TFA in HPLC grade water (10:90 v:v) at a flow of 1.5 ml $min^{-1}$. Phenacetin (rt = 4.8 min) was washed from the column following a step gradient to 25% acetonitrile.

Coumarin hydroxylation to umbelliferone (hydroxycoumarin) was used to assess CYP2A6 activity in human liver microsomes using methods modified from published procedures (11). Incubation mixtures of 0.5 ml contained 50 $\mu$g microsomal protein, 25 mM phosphate buffer (pH 7.4), 2.5 mM $MgCl_2$, 1 mM NADPH, and 50 $\mu$M coumarin. Reactions were stopped after 15 min by the addition of 0.5 ml 6% TCA (w/v). The samples were vortexed and then centrifuged to remove precipitated protein. A 0.5 ml aliquot of the supernatant was transferred to a tube containing 3 ml 0.8M Tris/0.8M glycine (pH 9.0). Umbelliferone concentrations in samples and standards were analyzed by fluorescence detection at excitation and emission wavelengths set at 380 nm and 460 nm, respectively.

Tolbutamide methyl-hydroxylation was used to assess CYP2C9 activity in human liver microsomes. Incubation mixtures of 0.5 ml contained 0.5 mg microsomal protein, 50 mM HEPES (pH 7.5), 15 mM $MgCl_2$, 1 mM $NADP^+$, 10 mM glucose-6-phosphate, 0.33 U glucose-6-phosphate dehydrogenase, and tolbutamide (100, 200, and 1000 $\mu$M). Reactions were stopped after 30 min by the addition of 25 $\mu$l 1N HCl. The samples were extracted by vortexing with 2 ml MTBE. After centrifugation, the organic layer was transferred to a clean tube and evaporated under nitrogen to dryness. Samples were reconstituted in HPLC mobile phase (47:53 methanol: 0.1% TFA in water, pH 2.6 with $NH_4OH$). The concentration of hydroxytolbutamide in each sample was determined by HPLC using a Zorbax SB-C8 column, 4.6 × 150 mm, and UV detection at 230 nm. At a flow rate of 1.5 ml $min^{-1}$, hydroxytolbutamide, chlorpropamide (internal standard), and tolbutamide eluted at approximately 3, 7, and 11 min.

CYP2D6 activity in human liver microsomes was assessed by determining dextrorphan formed from dextromethorphan using methods modified from published procedures (12). Incubation mixtures of 0.15 ml contained 50 $\mu$g microsomal protein, 50 mM HEPES (pH 7.5), 15 mM $MgCl_2$, 2 mM NADPH, and 100 $\mu$M dextromethorphan. Reactions were stopped after 15 min by the addition of a drop of 10% $NH_4OH$. The samples were extracted by vortexing with 1 ml MTBE. The MTBE layer was transferred after centrifugation to a clean tube and evaporated under nitrogen to dryness. Samples were reconstituted in HPLC mobile phase (25:75 acetonitrile: 30 mM ammonium acetate, pH 4.0). The concentration of dextrorphan in each sample was determined by HPLC using a Zorbax SB-C8 column, 4.6 × 150 mm, and UV detection at 230 nm. At a flow rate of 1.5 ml $min^{-1}$, dextrorphan and dextromethorphan eluted at approximately 3 and 10 min.

To determine their inhibitory effects on CYP3A activity, various concentrations of anastrozole, BMPN-b0enzoic acid, triazole, ketoconazole, or cimetidine were co-incubated *in vitro* as potential inhibitors with nifedipine using modified procedures from those described by Guengerich *et al.* (13). Incubation mixtures of 0.5 ml contained 0.1 mg microsomal protein, 50 mM HEPES (pH 7.5), 15 mM $MgCl_2$, 1 mM NADP+, 10 mM glucose-6-phosphate, 0.33 U glucose-6-phosphate dehydrogenase, and nifedipine (10, 25, and 50 $\mu$M). Reactions were stopped after 20 min by the addition of 0.1 ml $Na_2CO_3$. The samples were extracted by vortexing with 2 ml MTBE. The MTBE layer was transferred after centrifugation to a clean tube and evaporated under nitrogen to dryness. Samples were reconstituted in HPLC mobile phase (60:40 methanol:water). The concentration of dehydronifedipine in each sample was determined by HPLC using a Zorbax SB-C8 column, 4.6 × 250 mm, and UV detection at 260 nm. At a flow rate of 1.5 ml $min^{-1}$, dehydronifedipine and nifedipine eluted at approximately 6 and 9 min. Incubations, extractions, and HPLC analyses of samples and standards containing nifedipine were conducted while minimizing the exposure to light.

**Data Analysis.** Incubation of the CYP-marker substrates with microsomes from 1 to 3 individual donors in the presence or absence of various concentrations of anastrozole was used to determine $IC_{50}$ values. $IC_{50}$ values for the inhibition of the CYP activities were determined by nonlinear regression analysis (Origin, version 3.0, Microcal Software, Inc., Northampton, MA) using the following equation:

$$y = 100 - (100 - E_0) \times (I/(I + IC_{50}))$$

where I is the initial concentration of inhibitor in a microsomal incubation, $E_0$ is the per cent enzyme activity that is not inhibited at I = ∞ and, $IC_{50}$ is the inhibitor concentration that inhibits enzyme activity by 50%.

Rates of metabolite formation from pooled human liver microsomes following co-incubation of three concentrations of CYP-marker substrate with multiple inhibitor concentrations were used to determine $K_i$ values using the method of Dixon (14). The type of inhibition was assessed by transforming the data to give S/metabolite formation rate *vs.* inhibitor concentration (15).

## Results

The potential inhibitory effects of the aromatase inhibitor, anastrozole, on drug metabolism were evaluated by determining $IC_{50}$ and $K_i$ values for metabolic reactions that are selectively catalyzed by five different cytochrome P450 forms in human hepatic microsomes (table 1). Anastrozole inhibited less than 10% of the control coumarin

Downloaded from dmd.aspetjournals.org at ASPET Journals on July 20, 2018

TABLE 1

*Effects of anastrozole on the cytochrome P450 activities in human liver microsomes*

| CYP | Marker Substrate | Test Substance | $IC_{50}$ ($\mu$M)[a] | $K_i$ ($\mu$M) |
|---|---|---|---|---|
| 1A2 | Phenacetin | Anastrozole | 30 | 8/80 |
| 2A6 | Coumarin | Anastrozole | >500[b] | — |
| 2C9 | Tolbutamide | Anastrozole | 48 | 10 |
| 2D6 | Dextromethorphan | Anastrozole | >500[b] | — |
| 3A | Nifedipine | Anastrozole | 27 | 10/55 |
| 3A | Nifedipine | Triazole | >250[b] | — |
| 3A | Nifedipine | BMPN-benzoic acid | >250[b] | — |
| 3A | Nifedipine | Ketoconazole | 0.02 | — |
| 3A | Nifedipine | Cimetidine | 650 | — |

[a] $IC_{50}$ values were determined using the following concentrations of the specific CYP-marker substrates: phenacetin, 100 $\mu$M; coumarin, 50 $\mu$M; tolbutamide, 1 mM; dextromethorphan, 100 $\mu$M; nifedipine, 25 $\mu$M.

[b] Anastrozole and its metabolites (products formed by *N*-dealkylation) were not tested for CYP inhibition above 250–500 $\mu$M. Because little inhibition was observed at high concentrations, $K_i$ values were not determined.

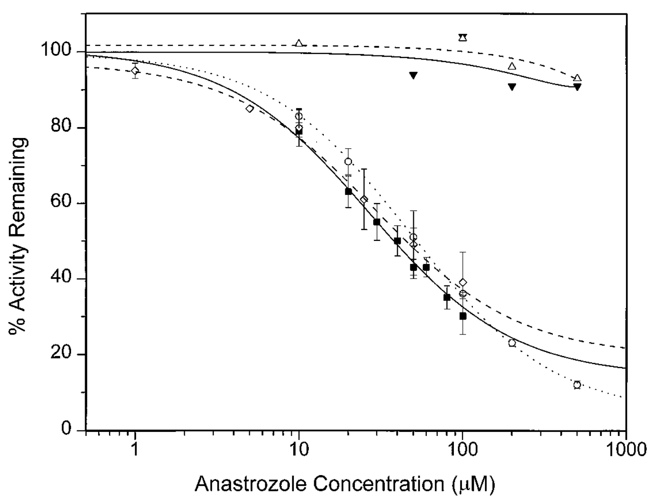


FIG. 2. *Inhibition of phenacetin O-deethylase (◇), coumarin hydroxylase (Δ), tolbutamide hydroxylase (○), dextomethorphan O-demethylation (▼), and nifedipine oxidation (■) in human liver microsomes by anastrozole.*

Data for phenacetin, tolbutamide, and nifedipine metabolism represent mean ± SE ($N$ = 3 individual microsome samples). Data for coumarin and dextromethorphan represent the average of duplicate incubations.

hydroxylase (CYP2A6) and dextromethorphan *O*-demethylase (CYP2D6) activities in the human microsome samples at concentrations up to 500 $\mu$M (fig. 2).

The *O*-deethylation of phenacetin as a marker activity for CYP1A2 was inhibited by anastrozole at concentrations between 10 and 100 $\mu$M with an $IC_{50}$ of 30 $\mu$M. Dixon plots of the reciprocal deethylation rates when 50, 100, or 200 $\mu$M phenacetin was co-incubated with 0 to 500 $\mu$M anastrozole exhibited considerable nonlinearity (fig. 3*A*).[2]

The nonlinearity in the results was also evident in the Cornish-Bowden plot (fig. 3*B*) when the data generated at 500 $\mu$M anastrozole

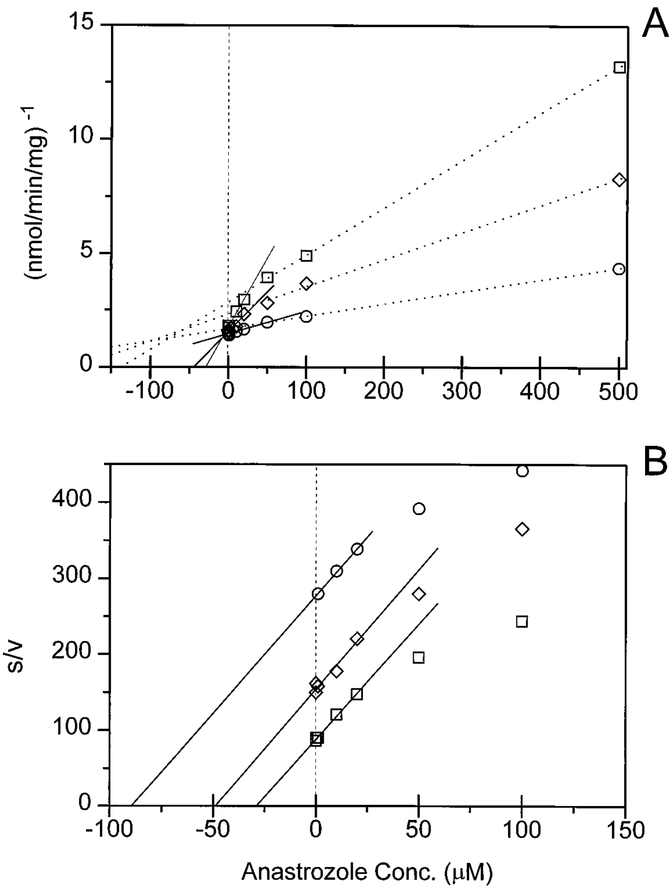


FIG. 3. *Dixon (A) and Cornish-Bowden (B) plots of the effect of anastrozole co-incubation on the rate of acetominophen formation from phenacetin in human liver microsomes.*

Phenacetin was incubated at initial concentrations of 50 (□), 100 (○), and 200 (◇) $\mu$M.

was excluded. The Dixon plot could be divided into two distinct linear phases. Therefore, when the data corresponding to the two phases were subjected to linear regression analysis, two apparent $K_i$ values of 8 and 80 $\mu$M were determined for the inhibition of phenacetin metabolism to acetaminophen. The high affinity $K_i$ of 8 $\mu$M estimated in this *in vitro* investigation was used to estimate conservatively the *in vivo* interaction potential towards CYP1A2 substrates. Parallel plots in the Cornish-Bowden transformation of the data indicate that the high affinity inhibition is competitive in nature.

Tolbutamide methyl-hydroxylase activity, a marker of CYP2C9 activity in human liver microsomes, was inhibited by anastrozole with an $IC_{50}$ of 48 $\mu$M when tolbutamide was co-incubated at 1000 $\mu$M (fig. 2). At this concentration of tolbutamide, CYP2C8 most likely contributes to the methylhydroxylase activity (16, 17) whereas at lower concentrations, the contribution by this CYP2C form would be negligible. An apparent $K_i$ of 10 $\mu$M was determined based on plots of the reciprocal hydroxylation rates when 100, 200, and 1000 $\mu$M tolbutamide was co-incubated with 0 to 100 $\mu$M anastrozole (fig. 4*A*). The inhibition of tolbutamide hydroxylase activity by anastrozole exhibited linear behavior on Dixon plots and Cornish-Bowden plots demonstrated parallel best-fit lines indicating competitive-type inhibition (fig. 5*B*).

Anastrozole inhibited nifedipine oxidation in human liver microsomes with an $IC_{50}$ of 27 $\mu$M. By comparison, ketoconazole was a

[2] The kinetics of phenacetin *O*-deethylation by the microsomes used in this investigation was best fit to a 2-enzyme model with a $K_m$ of 59 $\mu$M for the high affinity site. This result suggests that more than one enzyme contributes to this *O*-deethylation reaction and may potentially explain the appearance of biphasicity in the Dixon plot. In contrast and although other CYP3A enzymes are known to metabolize nifedipine, the kinetics of dehydronifedipine formation was fitted best to a 1-enzyme Michaelis-Menten model ($K_m$ ~25 $\mu$M).

Downloaded from dmd.aspetjournals.org at ASPET Journals on July 20, 2018

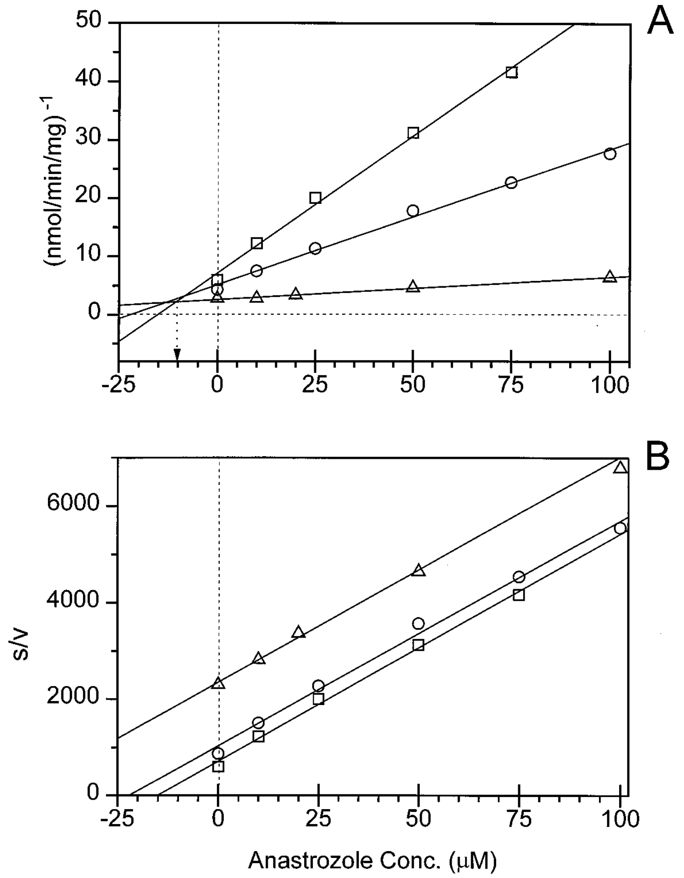


FIG. 4. *Dixon (A) and Cornish-Bowden (B) plots of the effect of anastrozole co-incubation on the rate of 4-hydroxytolbutamide formation from tolbutamide in human liver microsomes.*

Tolbutamide was incubated at initial concentrations of 100 (□), 200 (○), and 1000 (Δ) $\mu$M.

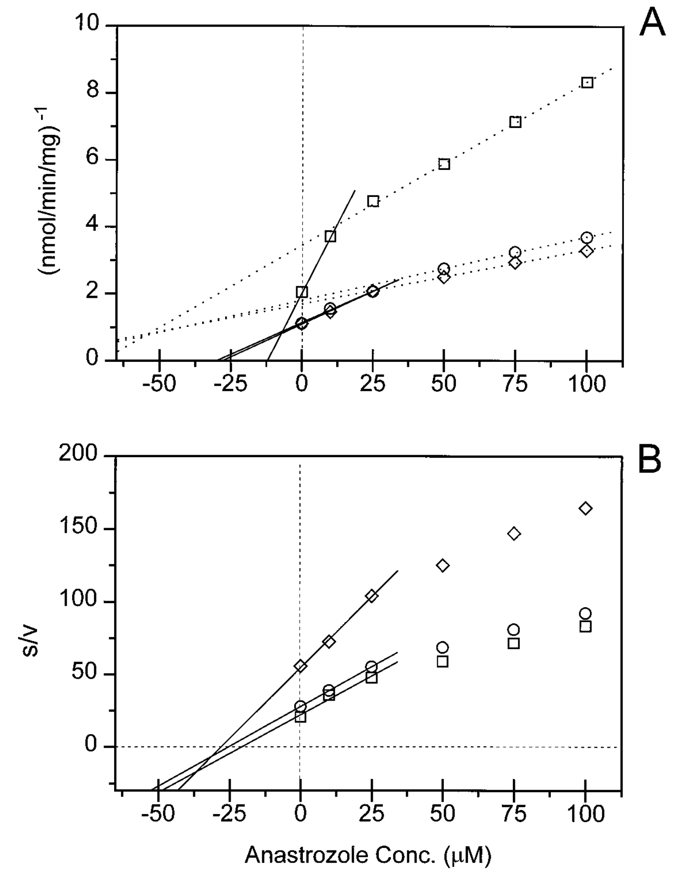


FIG. 5. *Dixon (A) and Cornish-Bowden (B) plots of the effect of anastrozole co-incubation on the rate of dehydronifedipine formation from nifedipine in human liver microsomes.*

Nifedipine was incubated at initial concentrations of 10 (□), 25 (○), and 50 ( 224 ) $\mu$M.

much more potent inhibitor of this CYP3A-catalyzed reaction with an $IC_{50}$ of 0.02 $\mu$M, while cimetidine was a weaker inhibitor ($IC_{50}$ = 650 $\mu$M) when co-incubated with nifedipine. Dixon plots of the reciprocal oxidation rates exhibited similar biphasicity to that observed for the inhibition of CYP1A2 by anastrozole (fig. 5*A*). Two distinct intercepts were obtained by linear regression analysis of the data and thus, two apparent $K_i$ values of 10 and 55 $\mu$M were determined for the inhibition of CYP3A-catalyzed nifedipine oxidation by anastrozole. Cornish-Bowden plots suggested that the CYP3A inhibition was mixed (competitive/noncompetitive) in nature (fig. 5*B*).

Because CYP3A4 appears to be the most abundant cytochrome P450 enzyme in both human liver and intestine and this enzyme is involved in the metabolism of many drug substrates, triazole and BMPN-benzoic acid were tested for their ability to inhibit nifedipine oxidation. These two compounds are major metabolites of anastrozole that are produced by *N*-dealkylation (cleavage of the methylene bridge). When co-incubated with nifedipine at concentrations up to 250 $\mu$M, these metabolites of anastrozole did not inhibit nifedipine oxidation.

## Discussion

Many, if not most, clinically relevant drug interactions are caused by inhibition of drug metabolizing enzymes leading to a decreased metabolic clearance and increased exposure to the inhibited drug. Cytochrome P450 enzymes mediate the rate limiting step of the primary Phase I metabolic reactions for the vast majority of drug compounds as well as the biosynthesis or catabolism of a number of endogenous substances with potent physiological and pathophysiological functions. Specific cytochrome P450 enzymes in the CYP1A, 2A, 2C, 2D, and 3A subfamilies are responsible for the oxidative metabolism of most drugs in humans (18, 19). Toxicities and other adverse events may therefore develop during clinical therapy with drugs that have narrow therapeutic margins when the concomitant administration of CYP inhibitors causes potent metabolic inhibition of drug clearance. Probably the most prominent example of this type of potentially harmful interaction is the cardiotoxicity that can be produced following co-administration of CYP3A4 inhibitors such as ketoconazole and erythromycin with terfenadine, a prodrug metabolized exclusively by CYP3A enzyme(s) (20). *In vitro* studies are now being used routinely to investigate the interaction potential for drugs resulting from inhibition of CYP-mediated metabolism (21, 22). The experiments reported here examined the ability of anastrozole to inhibit the metabolism of substrates for CYP1A2, CYP2A6, CYP2C9, CYP2D6, and CYP3A. The results were used 1) to compare the inhibition of drug metabolizing CYPs by anastrozole to its *in vitro* ability to inhibit estrogen synthesis mediated by human aromatase and 2) to predict the potential for anastrozole to cause metabolic drug interactions.

Anastrozole inhibited specific reactions catalyzed by CYP1A2, CYP2C9, and CYP3A but did not inhibit CYP2A6 or CYP2D6-mediated pathways. The inhibition of both CYP1A2 and CYP3A

Downloaded from dmd.aspetjournals.org at ASPET Journals on July 20, 2018

activities were biphasic in nature although the basis of the nonlinear results has not been established. Two apparent $K_i$ values were exhibited by anastrozole for inhibition of CYP1A2 and CYP3A metabolism. The low $K_i$ values for these two enzymes was similar to the single apparent $K_i$ determined for inhibition of CYP2C9 activity by anastrozole ($K_i = 8$ -10 $\mu$M corresponds to 2.3 to 2.9 $\mu$g ml$^{-1}$). The low $K_i$ values were used to estimate conservatively the potential for inhibitory drug interactions with anastrozole during clinical use of the drug. Average steady-state $C_{max}$ concentrations in patients chronically administered the 1-mg marketed dose of Arimidex were approximately 0.08 $\mu$g ml$^{-1}$ (0.3 $\mu$M). These plasma concentrations are 30-fold lower than the apparent $K_i$ values determined in these *in vitro* studies, suggesting that anastrozole would cause little or no inhibition *in vivo* of the metabolism of drugs that are substrates for CYP1A2, CYP2C9, and CYP3A enzymes (per cent inhibition *in vivo* predicted to be approximately 3%). Although a lack of inhibition potential can be predicted from these *in vitro* results, any effect of intracellular binding or accumulation of anastrozole in hepatocytes on the *in vivo* inhibition have not been accounted for in these investigations.[3]

Nifedipine oxidation in human liver microsomes was not decreased by two major metabolites (triazole and BMPN-benzoic acid) of anastrozole at concentrations up to 250 $\mu$M. The lack of CYP inhibition by these metabolites is consistent with requirement for both hydrophobic character for active site access and the heteratomic lone pair of electrons in triazole for Type II binding to the heme iron (23).

The results herein also show that the ability of anastrozole to inhibit drug metabolizing CYP enzymes in liver microsomes is much weaker than the inhibition of aromatase. When comparing the $IC_{50}$ for inhibition of human placental aromatase activity (15 nM) with the inhibition of the hepatic microsomal CYP produced by anastrozole *in vitro*, a margin of selectivity of at least 500-fold is obtained.

In conclusion, the results of the *in vitro* experiments performed in human liver microsomes indicate that, although anastrozole can inhibit CYP1A2, 2C9, and 3A-mediated catalytic activities, the level of inhibition *in vivo* during clinical therapy with Arimidex (Zeneca, Ltd., Macclesfield, UK) 1-mg would not be expected to cause clinically significant interactions with other CYP-metabolized drugs.

### References

1. P. V. Plourde, M. C. Dyroff, and M. Dukes: Arimidex®: A potent and selective fourth-generation aromatase inhibitor. *Breast Cancer Res. Treat.* **30,** 103–111 (1994).
2. P. E. Goss, and K. M. Gwyn: Current perspectives on aromatase inhibitors in breast cancer. *J. Clin. Oncol.* **12,** 2460–2470 (1994).
3. M. Murray and C. F. Wilkinson: Interactions of nitrogen heterocycles with cytochrome P-450 and monooxygenase activity. *Chem. Biol. Interact.* **50,** 267–275 (1984).
4. J. K. Ritter and M. R. Franklin: Induction and inhibition of rat hepatic drug metabolism by *N*-substituted imidazole groups. *Drug Metab. Dispos.* **15,** 335–343 (1987).
5. J. B. Houston, M. J. Humphrey, D. E. Matthew, and M. H. Tarbit: Comparison of two azole antifungal groups, ketoconazole and fluconazole, as modifiers of rat hepatic monooxygenase activity. *Biochem. Pharmacol.* **37,** 401–408 (1988).

[3] The plasma protein binding of anastrozole was determined *in vitro* to be 40% (unpublished results). Therefore, the free (unbound) concentration of anastrozole would be approximately 0.6 times the total concentrations in plasma.

6. D. J. Back and J. F. Tjia: Comparative effects of the antimycotic drugs ketoconazole, fluconazole, itraconazole, and terbinafine on the metabolism of cyclosporin by human liver microsomes. *Br. J. Clin. Pharmacol.* **32,** 624–626 (1991).
7. L. L. Von Moltke, D. J. Greenblatt, S. X. Duan, J. S. Harmatz, and R. I. Shader: In vitro prediction of the terfenadine-ketoconazole pharmacokinetic interaction. *J. Clin. Pharmacol.* **34,** 1222–1227 (1994).
8. R. G. Knodell, D. G. Browne, G. P. Gwozdz, W. R. Brian, and F. P. Guengerich: Differential inhibition of human liver cytochromes P-450 by cimetidine. *Gastroenterology* **101,** 1680–1691 (1991).
9. D. J. Newton, R. W. Wang, A. Y. H. Lu: Cytochrome P450 inhibitors: evaluation of specificities in the in vitro metabolism of therapeutic agents by human liver microsomes. *Drug Metab. Dispos.* **23,** 154–158 (1995).
10. J. R. Halpert, B. Naslund, and I. Betner: Suicide inhibition of rat liver cytochrome P-450 by chloramphenicol in vivo and in vitro. *Mol. Pharmacol.* **23,** 445–452 (1983).
11. J. S. Miles, A. W. McLaren, L. M. Forrester, M. J. Glancey, M. A. Lang, and C. R. Wolf: Identification of the human liver cytochrome P-450 responsible for coumarin 7-hydroxylase activity. *Biochem. J.* **267,** 365–371 (1990).
12. T. Kronbach, D. Mathys, and U. A. Meyer: High performance liquid chromatographic assays for bufuralol 1′-hydroxylase, debrisoquine 4-hydroxylase, and dextromethorphan *O*-demethylase in microsomes and purified cytochrome P-450 isozymes of human liver. *Anal. Biochem.* **162,** 24–32 (1987).
13. F. P. Guengerich, M. V. Martin, P. H. Beaune, P. Kremers, T. Wolff, and D. J. Waxman: Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J. Biol. Chem.* **261,** 5051–5060 (1986).
14. M. Dixon: The effect of pH on the affinity of enzymes for substrates and inhibitors. *Biochem. J.* **55,** 161–170 (1953).
15. A. Cornish-Bowden: A simple graphical method for determining the inhibition constants of mixed, uncompetitive, and non-competitive inhibitors. *Biochem. J.* **137,** 143–144 (1974).
16. J. A. Goldstein, M. B. Faletto, M. Romkes-Sparks, T. Sullivan, S. Kitareewan, J. L. Raucy, J. M. Lasker, and B. I. Ghanayem: Evidence that CYP2C19 is the major S-mephenytoin 4′-hydroxylase in humans. *Biochem.* **33,** 1743–1752 (1994).
17. M. E. Veronese, C. J. Doecke, P. I. Mackenzie, M. E. McManus, J.O. Miners, D. L. Rees, R. Gasser, U. A. Meyer, and D. J. Birkett: Site-directed mutation studies of human liver cytochrome P-450 isoenzymes in the CYP2C subfamily. *Biochem. J.* **289,** 533–538 (1993).
18. S. J. Wrighton and J. C. Stevens: The human hepatic cytochromes P450 involved in drug metabolism. *Crit. Rev. Toxicol.* **22,** 1–21 (1992).
19. M. Jurima-Romet, K. Crawford, T. Cyr, and T. Inaba: Terfenadine metabolism in human liver. In vitro inhibition by macrolide antibiotics and azole antifungals. *Drug Metab. Dispos.* **22,** 849–857 (1994).
20. S. Cholerton, A. K. Daly, and J. R. Idle: The role of individual human cytochromes P450 in drug metabolism and clinical response. *Trends Pharmacol. Sci.* **13,** 434–439 (1992).
21. B. J. Ring, S. N. Binkley, M. Vandenbranden, and S. A. Wrighton: *In vitro* interaction of the antipsychotic agent olanzapine with human cytochromes P450 CYP2C9, CYP2C19, CYP2D6, and CYP3A. *Br. J. Clin. Pharmacol.* **41,** 181–186 (1996).
22. G. N. Kumar, A. D. Rodrigues, A. M. Buko, and J. F. Denissen: Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. *J. Pharmacol. Exp. Ther.* **277,** 423–431 (1996).
23. B. Testa, and P. Jenner: Inhibitors of cytochrome P-450s and their mechanism of action. *Drug Metab. Rev.* **12,** 1–117 (1981).

Downloaded from dmd.aspetjournals.org at ASPET Journals on July 20, 2018