CYP1B1 Is Not a Major Determinant of the Disposition of Aromatase Inhibitors in Epithelial Cells of Invasive Ductal Carcinoma

Mostafizur Rahman, Sigurd F. Lax, Carrie H. Sutter, Quynh T. Tran, Gaylene L. Stevens, Gary L. Emmert, Jose Russo, Richard J. Santen, and Thomas R. Sutter

W. Harry Feinstone Center for Genomic Research (M.R., C.H.S., Q.T.T., G.L.S., T.R.S.) and Departments of Chemistry (M.R., G.L.E., T.R.S.) and Biology (M.R., C.H.S., Q.T.T., G.L.S., T.R.S.), University of Memphis, Memphis, Tennessee; Department of Pathology, General Hospital Graz West, Graz, Austria (S.F.L.); Fox Chase Cancer Center, Philadelphia, Pennsylvania (J.R.); and School of Medicine, University of Virginia, Charlottesville, Virginia (R.J.S.)

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ABSTRACT:

CYP1B1 and CYP19 (aromatase) have been shown to be expressed in breast tumors. Both enzymes are efficient estrogen hydroxylases, indicating the potential for overlapping substrate and inhibitor specificity. We measured the inhibition properties of aromatase inhibitors (AIs) against CYP1B1-catalyzed hydroxylation of 17β-estradiol (E2) to determine whether CYP1B1 affects the disposition of AIs. In addition, we estimated the frequency of coexpression of these enzymes in breast tumor epithelium. Immunohistochemical analyses of CYP19 and CYP1B1 in a panel of 29 cases of invasive ductal carcinoma of the breast showed epithelial cell staining for CYP19 in 76% and for CYP1B1 in 97% of the samples. Statistical analysis showed no significant correlation (0.33) for positive expression of CYP19 and CYP1B1 (p > 0.07).

CYP1B1 inhibition was determined for two steroidal inhibitors: formestane and exemestane and five nonsteroidal inhibitors: aminoglutethimide, fadrozole, anastrozole, letrozole, and vorozole. Of the seven compounds tested, only vorozole exhibited inhibition of CYP1B1 activity with IC50 values of 17 and 21 µM for 4-hydroxy estradiol and 2-hydroxy estradiol, respectively. The estimated Ki values of vorozole for E2 4- and 2-hydroxylation were 7.26 and 6.84 µM, respectively. Spectrophotometric studies showed that vorozole was a type II inhibitor of CYP1B1. This study shows that with the exception of vorozole, the aromatase inhibitors are selective for CYP19 relative to CYP1B1. Thus, although both CYP19 and CYP1B1 are expressed in a high percentage of breast cancers, CYP1B1 is not a major determinant of the disposition of AIs.

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ABBREVIATIONS: AI, aromatase inhibitor; E2, 17β-estradiol; PAGE, polyacrylamide gel electrophoresis; IDC, invasive ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor; miRNA, micro-RNA; 4-OHE2, 4-hydroxy estradiol; 2-OHE2, 2-hydroxy estradiol.
Muskhelishvili et al., 2001). CYP1B1 is the most catalytically efficient 17β-estradiol (E2) hydroxylase, preferentially producing 4-hydroxy-E2 (Hayes et al., 1996). Furthermore, CYP1B1 can oxidize the catechol estrogens to the chemically reactive semiquinone and quinone intermediates that form DNA adducts that can initiate breast, prostate, and other types of cancer (Cavaliere et al., 2006). Increased E2 4-hydroxylase activity has been measured in human breast cancer compared with normal breast tissue (Liehr and Ricci, 1996), and increased expression of CYP1B1 protein has been shown to occur in several types of human cancers, including breast and ovary (McFadyen et al., 1999).

Expression of CYP19 (Esteban et al., 1992; Brodie et al., 1997; Sasano et al., 2005; Oliveira et al., 2006; Miki et al., 2007) and CYP1B1 (McFadyen et al., 1999) proteins has been detected in breast cancer independently by immunohistochemistry, indicating that these enzymes are major tumor forms of cytochrome P450 expressed in cancer. Increased expression of CYP1B1 protein has been shown to occur in several types of human cancers, including breast and ovary (McFadyen et al., 1999).

Materials and Methods

Materials. Human CYP19 and human CYP1B1 microsomes were purchased from BD Gentest (Woburn, MA). The AIs obtained from multiple sources were kindly provided by Dr. William R. Miller (Western General Hospital, University of Edinburgh, Edinburgh, UK). Formentane, luteolone, fadrozole, and aminoglutethimide were obtained from Novartis (Basel, Switzerland). Exemestane was obtained from Pfizer (New York, NY). Anastrozole was obtained from AstraZeneca Pharmaceuticals LP (Wilmington, DE), and vorozole was from Janssen (Beerse, Belgium). The other materials used in this study were described previously in detail (Rahman et al., 2006).

CYP19 Protein Expression and Purification of Hexahistidine-Tagged Proteins in *Escherichia coli*. To generate fusion proteins, cDNA constructs were cloned into a vector engineered such that the expressed protein has an amino-terminal hexahistidine peptide tag to allow purification by metal chelate affinity chromatography. For the human CYP19 expression construct (herein referred to as His6-CYP19), a 730-bp PstI-KpnI fragment of the human aromatase cDNA (nucleotide positions 395-1125) (Corbin et al., 1988) was subcloned into the PstI-KpnI site of vector pTrcHis C (Invitrogen, Carlsbad, CA) and transformed into *E. coli* JM 109 cells. The plasmid was purified by alkaline lysis and the protein was expressed in *E. coli* transformants as described previously (Hayes et al., 1996).

The recombinant human CYP1B1 protein was purified by affinity chromatography with nitrolitriacetic acid-agarose (QiAGEN, Valencia, CA). Elution of His6-CYP1F9 from the nitrolitriacetic acid-agarose was achieved using a pH step gradient (pH 8.0, 6.3, and 4.5). Approximately 1 mg of affinity purified protein was then separated by SDS-PAGE using a 20-cm-long 12% polyacrylamide gel and stained with 0.3 M CuCl2 for 5 min. The area of the gel containing the fusion protein was excised using a razor blade and destained by repeated washing in 0.25 M EDTA and 0.25 M Tris.Cl, pH 9.0. The protein was eluted from the gel slice by electrophoresis in 25 mM Tris base and 192 mM glycine, pH 8.8, containing 0.1% SDS, then diaлизed against 0.5× phosphate-buffered saline and frozen at −20°C. The purity of the electroeluted protein was confirmed by Coomassie blue staining of the protein analyzed by SDS-PAGE.

Generation of Polyclonal Antibodies. All animal procedures were carried out by Spring Valley Laboratories (Sykesville, MD). A polyacrylamide gel slice containing 250 µg of purified fusion protein was minced and emulsified in complete Freund’s adjuvant and used for primary s.c. immunization of New Zealand White male rabbits. Rabbits were boosted 2 and 4 weeks following the primary immunization with 200 µg of purified protein, emulsified in incomplete Freund’s adjuvant. Serum was prepared from blood taken 5 weeks following the primary immunization. The serum IgG was purified by protein A affinity purification (Pierce Chemical, Rockford, IL; Walker et al., 1998).

Immunoblot Analysis. Microsomal samples were solubilized in SDS sample dilution buffer and separated by denaturing SDS-PAGE. Separated proteins were transferred to nitrocellulose (Hybond ECL; GE Healthcare, Little Chalfont, Buckinghamshire, UK) and incubated with primary antibody (anti-CYP19 at 5 µg/ml purified IgG and anti-CYP1B1 at 10 µg/ml; Walker et al., 1998) for 1 h at room temperature. Bound antibody was detected by incubation for 1 h with a horseradish peroxidase-linked secondary antibody (goat anti- rabbit IgG, 1:30,000 dilution; Promega, Madison, WI). Bound secondary antibody was detected by an enhanced chemiluminescence method according to the manufacturer’s instructions (Pierce).

Immunohistochemical Analysis. The purified serum IgG was used at a concentration of either 10 µg/ml (CYP19) or 10 µg/ml (CYP1B1). Nonspecific staining was assessed using normal rabbit serum (Sigma-Aldrich, St. Louis, MO) at a dilution of 1:5000. Paraffin-embedded human breast cancer samples including 29 cases of invasive ductal carcinoma (IDC), cases of cancer metastasis, and samples of normal breast tissue adjacent to cancer tissue were obtained as tissue microarrays from Imgenex (San Diego, CA; IMH-34640). Imgenex is the U.S. distributor for SuperBioChips (South Korea). Results for staining for estrogen receptor (ER), progesterone receptor (PR), and p53 were provided by Imgenex with the tissue arrays. The source of antibodies for these proteins was Dako Denmark A/S (Glostrup, Denmark; ER; M7047; PR, A0098; p53, M7001). Both the breast and placental tissues were obtained as clinical samples, without any identifiers. The slides were deparaffinized in xylene (twice for 4 min) followed by washing in 100% ethanol (4 min), 95% ethanol (2 min), 80% ethanol (15 s), and distilled water. Immunohistochemistry was performed as described previously (Walker et al., 1998), using diaminobenzidine as the chromagen. The slides were washed with distilled water for 1 min and counterstained for 1 min with hematoxylin. The intensities of the immunoreactivity were scored from 0 to 5, and the sample identifications were blinded from the pathologist (J.R.).

Statistical Analysis. Correlations between the epithelial cell expression of aromatase, CYP1B1, ER, PR, and p53 in IDC of the breast were determined using the Pearson χ2 test.

Inhibition Kinetics Assay Using the Microsomal Fraction Containing Recombinant Human CYP1B1. The recombinant human CYP1B1 protein was expressed in *Saccharomyces cerevisiae* as described previously (Hayes et al., 1996). Specific CYP1B1 protein content was estimated and evaluated by measuring the enzymatic activity as previously described (Rahman et al., 2006). For the initial AI screen, the same preparation of CYP1B1 microsomes was used for the inhibition studies. The measured E2 4-hydroxylase activity was 1.25 nmol/min/mmol P450, consistent with the turnover number published in our previous studies (Hayes et al., 1996; Rahman et al., 2006). The inhibition kinetics of CYP1B1 were determined in a range expected to produce 30 to 90% inhibition. A fixed substrate concentration and varying inhibitor
concentrations were used to determine the IC50 value at the point where 50% inhibition of the catalytic activity of the enzyme occurred. The E2 hydroxylation assay was performed with the addition of inhibitor and has been previously described in detail (Rahman et al., 2006). Inhibition was calculated as percentage of product formation compared with the corresponding control (enzyme-substrate reaction) without the inhibitors.

To determine the Ki value and type of inhibition from Dixon plots (Dixon, 1956), 10 pmol human CYP1B1 was assayed in the presence of three substrate concentrations: 1, 3, and 5 μM E2, and five concentrations of vorozole: 0.0, 5.0, 10.0, 20.0, and 40.0 μM, respectively. The substrate concentrations were approximately equal to 0.5 K_m, 2 × K_m, and 4 × K_m. The accurate values were 0.612 (0.5 × K_m), 2.45 (2 × K_m), and 4.89 (4 × K_m) μM. The concentration of vorozole was chosen and varied to give a wide range of percent inhibition based on our previously determined IC50 for this inhibitor. Linear regression analyses for each of the three substrate concentrations were plotted on a single graph of i/ rate versus inhibitor concentration. The rate of product formation was determined as picomoles of product per minute in each reaction. The coordinates of intersection of regression lines in Dixon plots are −Ki and 1/K_m. The Ki value is determined by averaging the results from individual intersections where the three regression lines did not intersect at a single point. Cornish-Bowden plot (SV/ against i) was also constructed using the same data to model the type of inhibition (Cornish-Bowden, 1974).

**Binding Difference Spectra of P450B1 with Vorozole.** The binding of vorozole to CYP1B1 was monitored by difference spectroscopy (Schenkman et al., 1967). The P450 content in microsomes containing recombinant human CYP1B1 expressed in the yeast S. cerevisiae was estimated to be 217.5 pmol/mg protein on the basis of reduced CO difference spectra. The microsomal protein was suspended in 0.1 M KPO4 buffer, pH 7.4. The diluted sample was transferred into both sample and reference cuvettes to give a protein concentration of 1 mg/ml and the baselines of equal light absorbance were recorded using a Varian Cary 100 Bio UV-Visible spectrophotometer (Varian, Inc., Palo Alto, CA) between 350 and 500 nm at ambient temperature. After the baseline had been recorded, vorozole dissolved in dimethyl sulfoxide was added to the sample cuvette in 2-μl aliquots and mixed gently with a pipette into the sample mixture. Vorozole was added to produce final concentrations of 5.0, 20.0, and 80.0 μM. An equal volume of carrier solvent was added to the reference cuvette. The sample was allowed to stand for 1 min, and the difference spectra were determined (350–500 nm) after each addition and graphed with baseline and background correction.

**Results**

**Expression of CYP19 Protein in E. coli and Production of Polyclonal Antibodies to CYP19.** For the generation of polyclonal antibodies to CYP19, we expressed CYP19 as hexahistidine-tagged fusion proteins in E. coli. High expression of His6-CYP19 was observed in protein extracts of induced E. coli cultures (Fig. 1A, lane 2). Metal chelate affinity purification yielded a 35-kDa CYP19 fusion protein, and this was confirmed by SDS-PAGE (Fig. 1A, lanes 4–7). Immunoblot analyses on the anti His6-CYP19 and anti His6-CYP1B1 antibodies showed that these antibodies reacted with their corresponding proteins in microsomes prepared from insect cells expressing recombinant human proteins. A single immunoreactive band of 60 kDa was detected by the anti-CYP19 antibody. Similarly, a single immunoreactive band of 56 kDa was detected by the anti-CYP1B1 antibody (Fig. 1B). The specificity of the anti-CYP19 antibody was shown by immunoblot analysis of protein fractions prepared from human placental tissue (Fig. 1C) and immunohistochemistry of human placental tissue (Fig. 1D). Only a single band of approximately 60 kDa was detected in either microsomes (Fig. 1C, lane 1) or the postmitochondrial supernatant (Fig. 1C, lane 2) prepared from two samples of human placenta. Immunohistochemistry demonstrated strong CYP19 staining in the syncytiotrophoblast of the placental villi (Fig. 1D, long arrow), with weaker staining in the cytotrophoblast and decidua (Fig. 1D). The strong staining of CYP19 in the syncytiotrophoblast of the placental villi has been reported for the characterization of other anti-CYP19 antibodies (Esteban et al., 1992). The sensitivity and specificity of the anti-CYP1B1 antibody has been reported previously (Walker et al., 1998; Kim et al., 2004). Representative immunohistochemical analyses of CYP19 and CYP1B1 expression in human breast invasive ductal carcinoma using the purified antibody are shown in Fig. 1C.

**Immunohistochemistry of Breast Cancer Using Anti-CYP19 and Anti-CYP1B1 Antibodies.** In this study, we evaluated the immunohistochemical expression of CYP19 and CYP1B1 in epithelial cells of breast cancer primarily with IDC. The tissue array we evaluated had 29 cases of IDC, as well as a small number of samples with metastatic carcinoma in lymph node (10 cases), infiltrating lobular carcinoma (two cases), ductal carcinoma in situ (two cases), singlet ring cell carcinoma (one case), solid papillary carcinoma (one case), and medullary carcinoma (one case). Table I reports the epithelial cell expression of CYP19 and CYP1B1 in cases of IDC of the breast. The results show a high percentage of expression of both CYP19 and CYP1B1 in epithelium of IDC (22 of 29). Similar results were observed for metastatic carcinoma in lymph node (seven of 10), infiltrating lobular carcinoma (two of two), ductal carcinoma in situ (one of two), singlet ring cell carcinoma (one of one), solid papillary carcinoma (one of one), and medullary carcinoma (one of one) (data not shown). CYP19 was positive in 22 cases of IDC (75.86%). Other tissue compartments (data not shown) expressing CYP19 included the blood vessels (13.79% positive) and fibroblasts (34.48% positive). CYP1B1 was expressed at high frequency and was positive in 28 (96.55%) cases of IDC. Both CYP19 and CYP1B1 were positive in 22 (75.86%) cases. ER, PR, and p53 were positive in 18 (62%), 11 (38%), and 12 (41%) cases, respectively. In addition to the IDC cases, we evaluated 10 samples of metastatic carcinoma in lymph node in epithelium for both CYP19 and CYP1B1 expression. We observed that CYP19 was positively expressed in lymph node in eight (80%) cases, whereas CYP1B1 was positively expressed in nine (90%) cases. Both CYP19 and CYP1B1 expression were positive in 70% cases (data not shown). To determine correlations between immunoreactivity of CYP19, CYP1B1, ER, PR, and p53, statistical analysis, using the Pearson χ2 test, was used. The correlation between the expression CYP19 and CYP1B1 was 0.33 and was not statistically significant (p > 0.07). Furthermore, no statistically significant correlations were found between immunoreactivity for CYP19 and the estrogen receptor, the progesterone receptor, or p53 status. Also, no statistically significant correlations were found using the Fisher’s exact test between CYP19 and CYP1B1 (data not shown).

The range of staining intensities of CYP19 and CYP1B1 were assigned scores of 0 to 5 according to the intensity of the staining for the expression of each enzyme. The expression of CYP19 in breast epithelial cells ranged from 0 to 4, with a median value of 1; the expression of CYP1B1 ranged from 0 to 5, with a median value of 3. We also determined the p value by Fisher’s exact test using staining intensities with the histologic grading 0 to 5 and found no correlation between immunoreactivity of CYP19 and CYP1B1 (data not shown).

**Inhibition of Human CYP1B1 Activity by AIs.** To explore inter-actions of CYP1B1 as a potential effector of the disposition of the AIs, the inhibition properties of seven aromatase inhibitors were evaluated for CYP1B1-catalyzed hydroxylation of E2. The chemical structures of steroidal and nonsteroidal aromatase inhibitors are shown in Fig. 2. The inhibition kinetics of steroidal (formestane and exemestane) and nonsteroidal (aminoglutethimide, fadrozole, anastrozole, letrozole, and vorozole) AIs are presented in Fig. 3. None of the AIs tested were potent inhibitors of human CYP1B1, except vorozole. The IC50 values for formestane, exemestane, androstenedione, aminoglutethimide,
fadrozole, anastrozole, and letrozole for human CYP1B1 E2 4- and 2-hydroxylation were found to be 100 μM. The IC50 values for vorozole for human CYP1B1 E2 4- and 2-hydroxylation were found to be 17 and 21 μM, respectively. Because of the observed lower IC50 values for vorozole compared with other AIs investigated, we determined the K_i value of vorozole. The Dixon plot was used to determine the dissociation constant, K_i, for the binding of inhibitor to enzyme and also to demonstrate the mechanism of inhibition of vorozole (Dixon, 1953). Figure 4, A and B, show Dixon plots for the inhibition of CYP1B1 by vorozole. The intersection of the regression lines in the upper left quadrant of a Dixon plot indicates competitive inhibition. The K_i values of vorozole for E2 4- and 2-hydroxylation were found to be 7.26 and 6.84 μM, respectively. It has been reported that for competitive inhibition kinetics, the K_i does not equal to IC50 (Cheng and Prusoff, 1973). We also observed here that IC50 and K_i values of vorozole for E2 4- and 2-hydroxylation are different. To provide further unambig-

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* Analysis by the Pearson χ² test.
expression of CYP19 and CYP1B1; the observed correlation was 0.33. The antibody raised against hexahistidine-tagged fusion protein of CYP19 was produced and purified from E. coli. We screened the AIs for inhibition of CYP1B1 because competitive inhibitors may also be enzyme substrates. Of the seven compounds tested, only vorozole, a triazole derivative, exhibited potent inhibition of human CYP1B1 activity. The other AIs studied, formestane, exemestane, aminoglutethimide, fadrozole, anastrozole, and letrozole, showed only weak inhibition of the catalytic activity of CYP1B1. The Dixon and Cornish-Bowden plots indicate that there is a competitive part in the inhibition of the human CYP1B1 enzyme by vorozole. Previously, this compound has been reported as a competitive inhibitor of aromatase (Montellano, 1995). Although vorozole inhibits human CYP1B1, this compound is over 1000-fold more active for aromatase inhibition (Wouters et al., 1994). It is unclear why other nonsteroidal inhibitors were not found to be as effective as vorozole in decreasing the catalytic activity of CYP1B1. These differences of action on CYP1B1 by nonsteroidal inhibitors may be due to the differences in their chemical structures. Although the inhibitory activities of AIs against human CYP1B1 have not been previously studied, the effect of tamoxifen, a nonsteroidal antiestrogen and a chemopreventive agent widely used for breast cancer treatment, was tested against human CYP1B1 as a possible inhibitor. However, the results of these studies were inconsistent (Rochat et al., 2001; Sridar et al., 2002). Rochat et al. (2001) have reported that tamoxifen reversibly inhibits CYP1B1 and is a noncompetitive inhibitor.

Discussion

Here, we report the production and characterization of a specific antibody raised against hexahistidine-tagged fusion protein of CYP19 that was produced and purified from E. coli. The expressions of CYP19 and CYP1B1 in cases of breast cancer were studied by immunohistochemical analysis. These analyses showed that a high percentage (76%) of the cases of IDC of the breast expressed both enzymes in the ductal epithelium. However, statistical analysis showed no significant correlation between the immunohistochemical expression of CYP19 and CYP1B1; the observed correlation was 0.33 (p > 0.07). Other characteristics of the tumor such as ER, PR, and p53 status did not show any statistically significant correlation with tumor aromatase. It should be noted that the number of cases of IDC evaluated in this study was small (n = 29). A recent paper in a population based study (n = 698) on hormonal markers in breast cancer identified a significant correlation (p < 0.01, Pearson correlation coefficient = 0.14) between CYP19 and CYP1B1 (Yang et al., 2007). We believe that the likely reason for the difference in results between this study and ours is sample size, with the larger study (Yang et al., 2007) having greater power to detect this correlation. In another study (Esteban et al., 1992), a significant (p = 0.04), but inverse correlation between CYP19 and ER immunoreactivity was reported, indicating a likelihood of detecting CYP19 in ER-negative tumors. A significant correlation between the expression of cyclooxygenase-2 and CYP19 in IDC and ductal carcinoma in situ from the same breast has also been reported (Oliveira et al., 2006). This group reported that 70% of the samples stained positive for CYP19 enzyme expression. In a recent study, two new CYP19 monoclonal antibodies were described and validated in an analysis of 43 cases of IDC (Sasano et al., 2005). Importantly, this latter study (Sasano et al., 2005) showed that there was a significant positive correlation between biochemical activity and CYP19 immunopositivity only in malignant epithelium.

CYP1B1 protein is overexpressed in malignant tumors of the breast (McFadyen et al., 1999). Interestingly, the elevated expression of CYP1B1 protein in breast cancer tissues has recently been shown to be regulated by micro-RNA (miRNA) expression (Tsuchiya et al., 2006). The researchers found that there was a significant inverse association between the expression levels of a specific miRNA (miR-27b) and CYP1B1 protein. It has been suggested that decreased expression of miR-27b would be one of the causes of the high expression of CYP1B1 protein in cancerous tissues. To our knowledge, the potential influence of miRNAs on the expression of CYP19 protein in tumor tissues has not been investigated.
However, in another independent study, Sridar et al. (2002) mentioned that tamoxifen had no effect on the activities of CYP1B1.

The binding of vorozole to human CYP1B1 resulted in type II spectra (Fig. 5), which indicates that this compound is likely associated with ligation of the heme iron (Miller, 2006). It has been reported that this type of binding arises from the nitrogen interaction with the iron atom. The chemical structures of type II compounds usually possess atoms with freely accessible nonbonding electrons, such as nitrogen lone pair or aromatic and aliphatic amines (Schenkman et al., 1967; Miller, 2006). It is to be noted that vorozole, a nonsteroidal type II inhibitor, like other nonsteroidal compounds, has basic nitrogen atoms that allows the inhibitors to interact reversibly with the heme prosthetic group, a common feature of all cytochrome P450 enzymes (Johnston and Dowsett, 2003). Thus, vorozole is unlikely to be a substrate for CYP1B1. Preliminary studies with microsomal incubation of CYP1B1 with vorozole indicate no metabolic products as determined by high-performance liquid chromatography with UV/Vis (data not shown).

AIs have been proposed for the prevention of breast cancer. In adjuvant therapy trials comparing tamoxifen with AIs, the reduction of contralateral breast cancer is 50% greater with the AI than with tamoxifen in separate trials (Kudachadkar and O'Regan, 2005). It has been postulated that the greater efficacy of the AIs is because of the reduction of estrogen levels and therefore their genotoxic metabolites. Vorozole reduced plasma levels of E2 in premenopausal women to ~36% after 8 h of intake (Wouters et al., 1989). It was also a more effective estrogen suppressant, resulting in 30% more suppression of serum E2 levels in postmenopausal women than formestane, a steroidal inhibitor (Dowsett et al., 1999). However, vorozole has not been approved for use by the U.S. Food and Drug Administration, although its clinical activity was similar to anastrozole and letrozole in phase III trials (Hamilton and Piccart, 1999). It has been reported that at clinically administered doses, the plasma half-lives of anastrozole (1 mg/day), letrozole (2.5 mg/day), and exemestane (25 mg/day) are 41 to 48 h, 2 to 4 days, and 27 h, respectively (Buzdar, 2003). There are some differences in the degree of plasma estradiol suppression among the 3rd generation AIs. The three aromatase inhibitors

Fig. 3. Inhibition of human CYP1B1 by aromatase inhibitors. Microsomes containing 10 pmol CYP1B1 were incubated with 3 μM E2 and 0.0, 1.0, 10.0, and 100 μM each aromatase inhibitors in a single determination. Reactions were run for 15 min at 37°C, and rates of 4-OHE2 (hatched bar) and 2-OHE2 (open bar) formation were determined as described under Materials and Methods.
anastrozole, letrozole, and exemestane, that are currently approved by the Food and Drug Administration for use in breast cancer treatment, decrease estradiol by 84.9, 87.8, and 92.2%, respectively (Osborne and Tripathy, 2005). The 3rd generation AIs are well tolerated and suppress endogenous estrogen levels in postmenopausal women, but estrogen deficiency is one of the most important factors for the pathogenesis of osteoporosis. Osteoporotic fractures and greater bone mineral loss are typically more associated with AIs (Osborne and Tripathy, 2005). Based on the results of this study, it is indicated that with the exception of vorozole, the AIs are specific for aromatase relative to CYP1B1, a cytochrome P450 widely expressed in breast tissue and responsible for estrogen metabolism. Overall, the results of this study indicate that CYP1B1 is not a major determinant of the disposition of AIs.

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References


