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Biotransformation of letrozole in rat liver microsomes: Effects of gender and tamoxifen

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Abstract

The *in vitro* metabolic kinetics of letrozole were investigated by incubating letrozole (10–500 μM) in female or male rat liver microsomes to assess the effect of gender and to predict the *in vivo* biotransformation characteristics of letrozole in rats. The effects of tamoxifen (TAM) on the metabolic kinetics of letrozole were also examined by incubating letrozole in female rat liver microsomes in the presence or absence of TAM. The effects of chronic pretreatment of female rats with TAM (0.5, 1.0, 5.0 mg/kg/day, *i.p.* for 7 consecutive days) on liver microsomal protein content and metabolic activity were also examined.

The formation rate of the carbinol metabolite of letrozole, CGP44 645, was significantly higher ($p < 0.05$) in male rat liver microsomes in comparison to female. The V_{max}/K_m ratio for letrozole metabolism in female rat liver microsomes did not change significantly ($p > 0.05$) in the presence of TAM. After chronic pretreatment of female rats with TAM (up to a dose of 1.0 mg/kg/day), the hepatic microsomal protein content was significantly increased but the formation rate of CGP44 645, when normalized for protein content, did not change significantly.

These results suggest that there is a marked gender difference in letrozole metabolism in rats. It also appears that acute treatment of female rat liver microsomes with TAM produces negligible inhibitory effect on the CYP mediated metabolic clearance of letrozole. However, chronic pretreatment of female rats with TAM appear to induce CYPs, but does not significantly impact the metabolic activities of the enzymes associated with the formation of the carbinol metabolite of letrozole.

Keywords: Metabolism; Liver microsomes; Letrozole; Tamoxifen; Gender

1. Introduction

About 75% of breast cancers are positive for the estrogen receptor (ER) and/or progesterone receptor (PgR), and estrogen is the main stimulant in the development and growth of these tumors [1]. Therefore, estrogen deprivation is the primary mechanism of hormonal therapies for breast cancer.

There are two main ways in which estrogen deprivation may be achieved (Fig. 1). The most frequent approach is to use an antiestrogen such as tamoxifen (TAM, Fig. 2) to antagonize estrogens at the estrogen receptor (ER). TAM is now recommended for all pre-menopausal women with hormone-positive cancers, as well as for most postmenopausal women with breast cancer and/or a growing number of women with hormone-negative cancers [2,3]. However, the actions of TAM are complex. For example, it also has partial estrogen-agonist effects. These partial agonist effects can be beneficial, since they may help prevent bone demineralization in postmenopausal women [4,5], but also detrimental, since they are associated with increased risks of secondary cancer, such as uterine and/or liver cancer [6–8] and thromboembolism [9]. In addition, not all patients with advanced ER-positive disease respond to TAM and nearly all of those who do respond eventually relapse with resistant disease [1]. Studies over many years have indicated that the partial agonist effect of TAM may play a part in the development of TAM resistance [10].

An alternative approach is to use an aromatase inhibitor (AI) such as letrozole to reduce the synthesis of estrogens. The aromatase is a cytochrome P450 enzyme that converts adrenal androgens such as testosterone and androstenedione to estrogens such as estradiol and estrone, respectively. Letrozole (Fig. 2) is a

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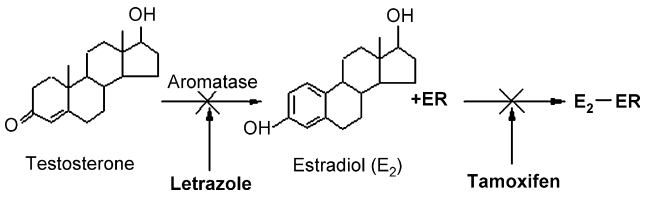

Fig. 1. Inhibition of aromatase by letrozole and antagonism of estrogen receptor (ER) binding by tamoxifen.

nonsteroidal, highly potent and competitive inhibitor of the aromatase enzyme system. It has been reported that the treatment of women with letrozole significantly lowers serum estrone (E_1), estradiol (E_2) and estrone sulfate (E_1S) by 88–98% [11], but produces no androgenic, progestrogenic or estrogenic effects, such as weight gain, acne, or hypertrichosis [12]. Therefore, it is used as a second-line agent (after TAM) and now challenging the place of TAM for the treatment of advanced breast cancer in postmenopausal women.

The differences in the mechanism of action between letrozole and tamoxifen have led to suggestions that a combination of the two agents may produce a better outcome in breast cancer treatment compared to either agent alone. However, a combination of letrozole and tamoxifen also raises the possibility of potential metabolic interaction between the two drugs since both compounds are substrates for cytochrome P450 (CYPs). It has been known that subfamilies of CYP3A4 and CYP2A6 are responsible for the metabolism of letrozole to its carbinol metabolite, CGP44 645, in humans [13]. However, no account of the metabolic kinetics of letrozole either in animal or human models has been reported. Therefore, our first goal was to obtain metabolic kinetic parameters of letrozole in rat liver microsomes, in an attempt to predict the *in vivo* clearance and other pharmacokinetic characteristics of letrozole in rats. Interestingly, hepatic CYP3A4 is the major subfamily responsible for the metabolism of TAM as well [14], indicating that letrozole and TAM are both substrates of CYP3A4. Therefore, there is a possibility of metabolic interaction between these two drugs as a result of enzyme inhibition or induction. Previous studies have shown that acute treatment of rats with TAM increased hepatic CYP2A1 activity and decrease CYP2C11 activity [15], while pretreatment with TAM induced CYP2B1 and CYP3A1 in rat liver microsomes [16]. However, it has also been observed that TAM and its metabolites reversibly inhibit CYP3A4 and CYP1B1 in human liver microsomes [17]. Nevertheless, there is little information regarding metabolic interaction between letrozole and TAM in the literature. Therefore, it is necessary to investigate the impact of TAM on the metabolic kinetics of letrozole.

The objective of this investigation was to evaluate the *in vitro* metabolic kinetics of letrozole and examine gender differences and the effect of TAM treatment on the biotransformation of letrozole in rat liver microsomes. The information generated may be useful to predict the *in vivo* intrinsic clearance of letrozole and metabolic interaction between letrozole and tamoxifen in animals.

2. Experimental

2.1. Materials

Letrozole was generously provided by Dr. A. Brodie (University of Maryland, School of Medicine, Baltimore, MD, USA), and its carbinol metabolite CGP44 645 was synthesized by Drs. Piao and Canny (Temple University School of Pharmacy, Philadelphia, PA). Methanol, acetonitrile, monobasic (and dibasic) sodium phosphate, and potassium chloride (KCl) were purchased from Fisher Scientific (Pittsburg, PA, USA). Tamoxifen, tamoxifen citrate salt, β-nicotinamide adenine dinucleotide (β-NADP), glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PD), bovine serum albumin, phosphate buffer saline tablets and hydroxypropyl-β-cyclodextrin (HPβCD) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). BioRad reagent was ordered from BIO-RAD Laboratories (Richmond, CA, USA). All chemicals and solvents were of analytical or HPLC grade. Distilled water was obtained from an in-house Barnstead NANOpure® apparatus (Barnstead International, Dubuque, IA, USA). Bond Elut-C_8® extraction cartridges were ordered from Varian (Walnut Creek, CA, USA).

2.2. Preparation of rat liver microsomes

Male and Female Sprague–Dawley rats (300 ± 20 g) were obtained from Charles River Laboratories (Wilmington, MA, USA). The animals were maintained in a controlled environment of constant temperature (20 °C), 50% relative humidity and 12-h light:12-h dark cycles for 7 days prior to use. The rats were provided with food and water *ad libitum*. The health of all animals was monitored throughout the study by observing the changes in body weights.

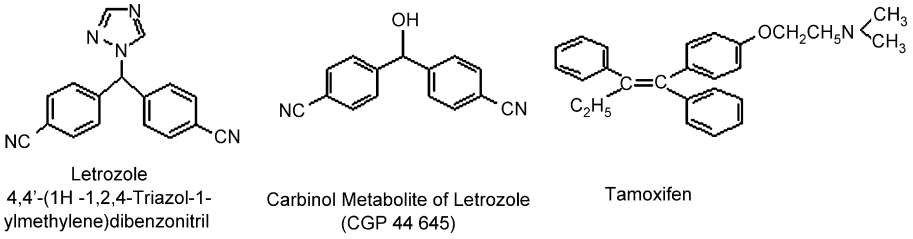

Fig. 2. Structures of letrozole, its carbinol metabolite, CGP 44645, and tamoxifen.