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Regular article

Comparative metabolic study between two selective estrogen receptor modulators, toremifene and tamoxifen, in human liver microsomes☆

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ABSTRACT

Toremifene (TOR) and Tamoxifen (TAM) are widely used as endocrine therapy for estrogen receptor positive breast cancer. Poor metabolizers of TAM are likely to have worse clinical outcomes than patients who exhibit normal TAM metabolism due to lower plasma level of its active metabolite, 4-hydroxy-*N*-desmethyl (4OH-NDM) tamoxifen (endoxifen). In this study, we examined the role of individual cytochrome P450 (CYP) isoforms in the metabolism of TOR to *N*-desmethyl (NDM), 4-hydroxy (4OH) and 4OH-NDM metabolites in comparison with TAM using human liver microsomes (HLMs) with selective chemical inhibitors for each CYP isoform and recombinant CYP proteins. Similar levels of NDM metabolites were formed for both TOR and TAM, and *N*-demethylation of both compounds was primarily carried out by CYP3A4. We found that the formation of 4OH-NDM-TOR was catalyzed both by CYP2C9 and CYP2D6, whereas the formation of 4OH-TAM and endoxifen was specifically catalyzed by CYP2D6 in HLMs. Our results suggest that the potential contribution of CYP2D6 in the bioactivation pathway of TOR may be lower compared to TAM, and may have a different impact on clinical outcome than *CYP2D6* polymorphisms.

1. Introduction

Tamoxifen (TAM) is widely used as a selective estrogen receptor modulator (SERM) for estrogen receptor positive breast cancer [1]. Toremifene (TOR) has a structure that is similar to TAM, and has also been approved as a treatment for recurrent and advanced breast cancer and postoperative adjuvant endocrine therapy [2,3]. However, recent studies have suggested wide variability in the level of active metabolites of TAM in plasma that could affect both efficacy and toxicity of the drug. Such differences are in part based on genetic polymorphisms in the cytochrome P450 (CYP) metabolic system [4–8] as well as individual patient factors which have an influence on pharmacokinetic variations in general e.g., regulating function of drug absorption and disposition [9], and drug–drug interactions in use of concomitant drugs [10,11].

The main metabolic pathway of TAM has been investigated from a number of *in vitro* and *in vivo* studies (Fig. 1). Demethylation of the aminoethoxy side chain of TAM to *N*-desmethyl-TAM (NDM-TAM) appears to be the main route of TAM metabolism in humans [10]. 4-Hydroxy-TAM (4OH-TAM) is a relatively minor metabolite, but has been shown to have a high affinity for ERs and a 100-fold higher potency than TAM and NDM-TAM in suppressing estrogen-dependent cell proliferation [12,13]. 4-Hydroxy-*N*-desmethyl-TAM (endoxifen), a secondary metabolite of TAM, exhibits potency similar to 4OH-TAM with respect to ER binding affinity and suppression of estrogen-dependent cell growth [14]. Endoxifen is likely to bestow the greatest contribution to clinical efficacy due to a much higher (>6-fold) plasma concentration in breast cancer patients compared to 4OH-TAM [10].

From *in vitro* studies using human liver microsomes (HLMs), TAM is known to be metabolized by several CYP isoforms—NDM-TAM formation is mainly catalyzed by CYP3A4, and sequentially, endoxifen formation is mainly catalyzed by CYP2D6 [15–17]. Recently, it has been suggested that *CYP2D6* genotypes can

Abbreviations: TOR, toremifene; TAM, tamoxifen; SERM, selective estrogen receptor modulator; SSRI, selective serotonin reuptake inhibitor; 4OH, 4-hydroxy; NDM, *N*-desmethyl; 4OH-NDM, 4-hydroxy-*N*-desmethyl; CYP, cytochrome P450; HLM, human liver microsome; LC-MS/MS, liquid chromatograph-tandem mass spectrometry; SRM, selective reaction monitoring; CL_{int}, intrinsic clearance.

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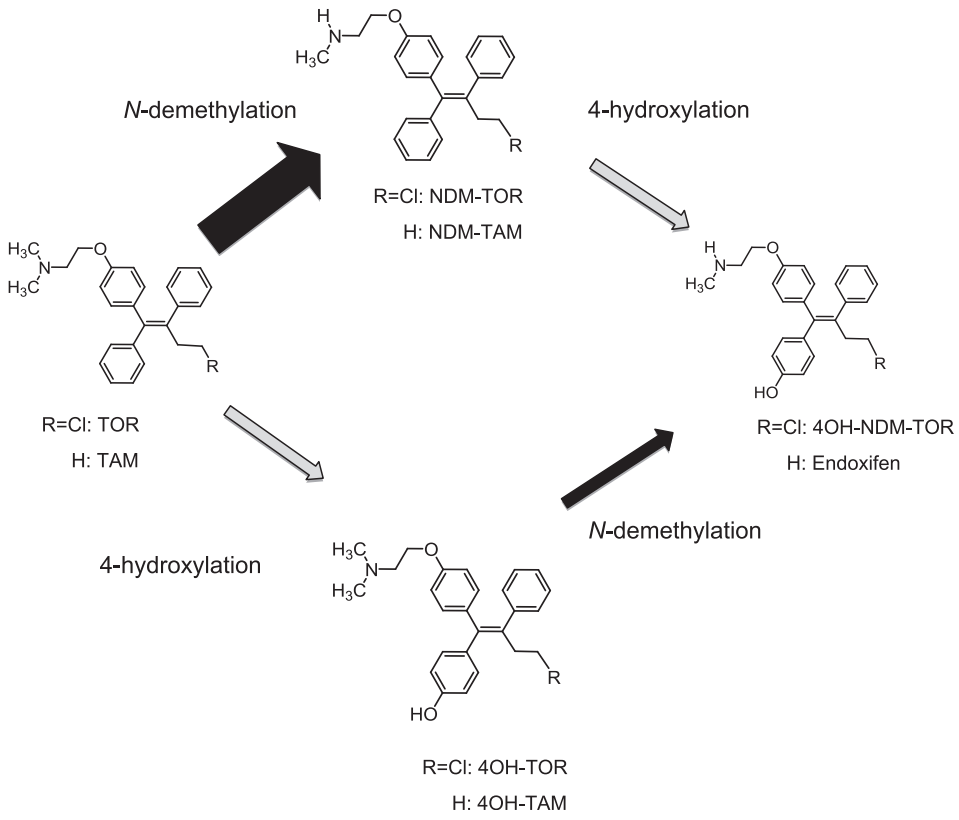

Fig. 1. Metabolic pathway of TOR and TAM in humans. Based on plasma concentration, the main metabolites of TOR and TAM are *N*-desmethyl-metabolites, while their 4-hydroxy-metabolites are at much lower levels in human plasma. The secondary metabolites of TOR and TAM, 4OH-NDM-TOR and endoxifen, are produced first by *N*-demethylation followed by 4-hydroxylation.

influence endoxifen steady-state plasma concentration, and thus the clinical benefits of TAM as well.

A number of clinical trials have reported the association between the *CYP2D6* genotype and clinical outcome of breast cancer patients on TAM therapy [4–7,9,18–20]. One of the first studies reported in 2005 demonstrated that homozygous carriers of *CYP2D6*4* (non-functional allele) had shorter relapse-free survival and disease-free survival compared with patients who were heterozygous or homozygous for the wild-type allele [4]. In Asians, it has been reported that *CYP2D6*10* (decreased functional allele) is significantly associated with worse clinical outcome in Japanese [9,20] and Chinese patients [7]. Additionally, use of selective serotonin reuptake inhibitors (SSRI), e.g., paroxetine, during TAM treatment reduces endoxifen levels [10] and is associated with an increased mortality risk from breast cancer [11].

Indeed, the relationship between *CYP2D6* polymorphisms and therapeutic effects of tamoxifen has been studied for a decade, but the results are actually contradictory and inconclusive because there is no conclusive evidence of relationship between endoxifen concentrations according to *CYP2D6* genotypes and clinical response.

Like TAM, TOR also undergoes biotransformation to NDM-, 4OH- and 4OH-NDM-metabolites *in vitro* (Fig. 1). However, it is unclear which enzymes are involved in catalyzing the conversion of TOR to these active metabolites. A previous study using human recombinant P450 microsomes showed the likely involvement of CYP3A4 and CYP2D6 in primary and secondary *N*-demethylation of TOR and 4OH-TOR, and that CYP2D6 and CYP2C19 may play an important role in the 4-hydroxylation metabolism of TOR and NDM-TOR [21]. However, the specific contributions of each of the other CYP isoforms to TOR metabolism remain unclear. Previous studies have shown that the main metabolite of TOR in human plasma is NDM-TOR, while levels of 4OH-TOR and 4OH-NDM-TOR are much lower or undetectable in plasma [21,22]. The latter two metabolites, however, have been reported to be detected in human urine samples at a higher dosing (a single 120 mg oral dose) [23], and it has been suggested that these potent metabolites are likely to contribute primarily or partially to antitumor effects due to their extremely high growth inhibition activities, particularly when high-dose TOR therapy is given.

In this study, we identified CYP isoforms associated with TOR metabolism in human recombinant P450 microsomes and HLMs using isozyme selective inhibitors. Additionally, we assessed the inhibitory effects of paroxetine on NDM-TOR 4-hydroxylation to confirm whether the CYP2D6 isoform contributes to metabolic activation. Indeed, intrinsic clearance of 4-hydroxylation of NDM-TOR was estimated in HLMs with intermediate or lower CYP2D6 activities. Given the important differences between TOR and TAM metabolic biotransformation in human cells *in vitro*, our studies should allow improved understanding of the mechanisms and factors that control TOR activation, and also support the expectation that drug–drug interactions or pharmacogenomics related to CYP2D6 are unlikely to influence TOR's clinical benefit.

2. Methods

2.1. Chemicals

Toremifene citrate, *N*-desmethyltoremifene citrate, 4-hydro xytoremifene, 4-hydroxy-*N*-desmethyltoremifene and internal standard (structural analog of TOR) were provided by Orion Corporation (Espoo, Finland). Tamoxifen citrate was purchased