CYP2D6 and Tamoxifen Metabolism

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Tamoxifen is an important adjuvant endocrine therapy in estrogen receptor (ER)-positive breast cancer patients. It is metabolized into its most active antiestrogenic metabolite endoxifen, predominantly by cytochrome P450 2D6 (CYP2D6). Many factors, including genetic variation in CYP2D6, influence tamoxifen metabolism and pharmacokinetics.

tamoxifen | Pharmacogenetics | PGx | CYP2D6 | Cytochrome P450 2D6 | endoxifen | tamoxifen metabolism | tamoxifen pharmacokinetics

1. Introduction

For many years, tamoxifen has been known as the most important adjuvant endocrine treatment in patients with estrogen receptor (ER) positive breast cancer [1][2]. It is a selective estrogen receptor modulator (SERM), which inhibits tumor growth and promotes apoptosis in ER-positive tumors [3], resulting in a reduced risk of recurrence and death from breast cancer [4]. Tamoxifen is metabolized into its most active antiestrogenic metabolite endoxifen, predominantly by cytochrome P450 2D6 (CYP2D6). Despite high efficacy, a wide variability in the response of individuals to standard doses of tamoxifen is seen [4]. Factors influencing drug responses such as gender, age, obesity, drug–drug interactions, drug–food interactions, comorbidity, liver and renal function, pregnancy, circadian rhythm, as well as genetic factors could possibly explain this wide variability [5][6][7][8][9][10].

As genetic variation in *CYP2D6* leads to altered enzyme activity and thereby potentially to an affected efficacy [5], pharmacogenetic (PGx) testing could play a major role in optimizing tamoxifen treatment. Nowadays, PGx testing is available for an increasing number of drugs, mainly in psychiatric but also for cardiologic and oncologic applications. However, only a small number of drugs are considered to require upfront genotyping, such as *HLA-B*5701* genotyping for abacavir, *CYP2C19* genotyping for clopidogrel, and *DPYD* testing for fluoropyrimidines treatment [11][12][13]. The challenge of PGx testing is to obtain actionable information of genetic variants and their influence on outcome. Experts differ in their interpretation of published evidence and their recommendations [5]. This also applies to tamoxifen. Whereas the first studies on *CYP2D6* genotyping for optimizing tamoxifen therapy based on pharmacokinetic studies [14] or outcome [15][16] were published in 2005, its clinical implementation is still being debated [17][18]. The controversy was most prominently seen in 2012. At that time, two studies were published, and both concluded there was no significant association between *CYP2D6* genotype and outcome in breast cancer patients treated with adjuvant tamoxifen therapy [19][20]. This triggered several researchers with opposite visions, leading to a correspondence as published in the JNCI [21][22][23][24]. In 2013, several meta-

analyses were performed in order to answer the question of whether *CYP2D6* status affects clinical outcomes in tamoxifen therapy [25][26][27][28][29]. However, also these findings yielded contradictory results. In 2018, the Clinical Pharmacogenetics Implementation Consortium (CPIC) published a recommendation on *CYP2D6* genotyping for guiding adjuvant tamoxifen therapy, thus supporting the importance of the *CYP2D6* genotype in tamoxifen therapy. However, the European Society for Medical Oncology (ESMO) indicated in a publication in 2019 that according to their view, there was no place for *CYP2D6* genotyping in a clinical setting [18].

According to predictions, over 3 million women will be diagnosed with breast cancer in 2040 [30], which triggers the strong need for optimizing tamoxifen treatment.

2. Tamoxifen Metabolism

Tamoxifen is a prodrug, which is converted into multiple derivatives by phase I enzymes. Among these, 4-hydroxytamoxifen (4OH-TAM) and endoxifen show the strongest affinity with the ER [2][31]. As the plasma concentration of endoxifen is 6 to 12 times higher compared with 4OH-TAM, and endoxifen has the lowest IC50 at the ER, endoxifen is considered the major active tamoxifen metabolite [2][31]. Tamoxifen is *N*-demethylated, predominantly by CYP3A4 and CYP3A5, resulting in inactive *N*-desmethyltamoxifen (NDM-TAM) (Figure 1). 4-hydroxylation of NDM-TAM is nearly exclusively performed by CYP2D6, resulting in the formation of 4-hydroxy-*N*-desmethyltamoxifen, also known as endoxifen [2][31]. As shown in Figure 1, demethylation and hydroxylation can also occur in the opposite order, resulting a different intermediate metabolite called 4-hydroxytamoxifen (4OH-TAM) [2][31]. As NDM-TAM is the primary metabolite regarding plasma concentrations, the demethylation followed by hydroxylation is supposed to be the main route [32].

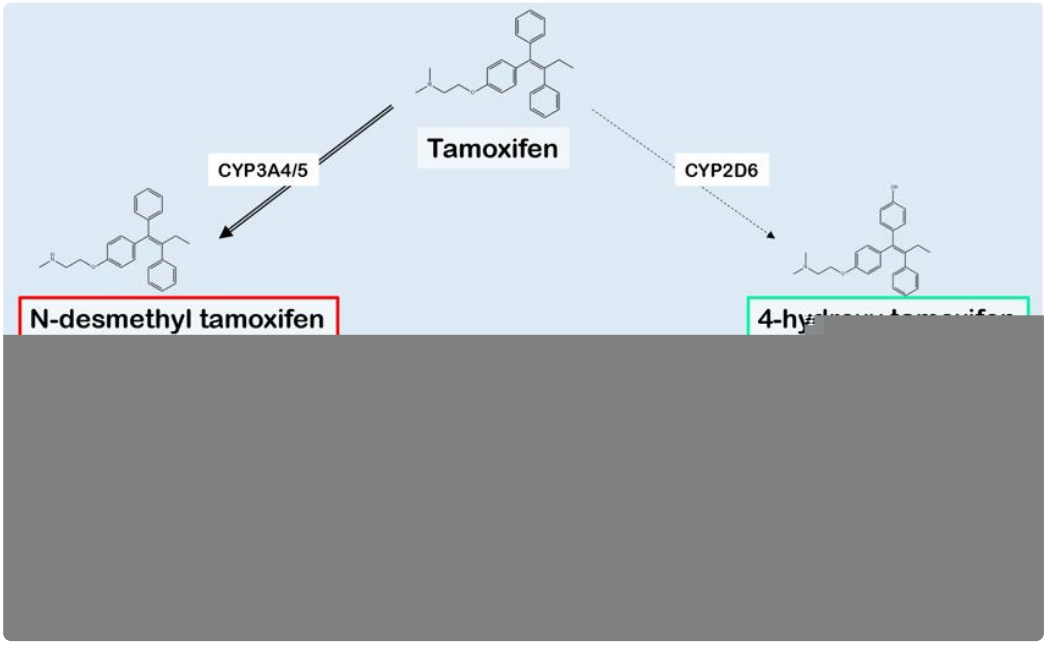

Figure 1. Simplistic representation of the main biotransformation of tamoxifen and its metabolites. The generation of N-desmethyltamoxifen (NDM-TAM) is predominantly catalyzed by CYP3A4/5, whereas especially CYP2D6 is

responsible for the formation of 4-hydroxytamoxifen (4OH-TAM) and endoxifen. The activity of metabolites is shown using red to indicate for inactivity and green for activity. The various metabolites are inactivated by UGTs and SULTs, mainly isoform SULT1A1. Abbreviations: CYP: Cytochrome P450 isoenzymes, UGT: UDP-glucuronosyltransferase, SULT: sulfotransferase isoenzyme. Figure based on Jin, et al. [14].

3. Cytochrome P450 2D6

CYP2D6 is involved in the metabolism of ≈25% of the most commonly used drugs, whereas it only accounts for approximately 2% of total liver CYP protein capacity [2][33]. As shown in Figure 1, CYP2D6 is responsible for the specific conversion of NDM-TAM to endoxifen [31]. Therefore, CYP2D6 is considered the most important drug-metabolizing enzyme in tamoxifen metabolism. The *CYP2D6* gene is located on chromosome 22q13.2 and is highly polymorphic [34]. Currently, approximately 150 single nucleotide polymorphisms (SNPs) and 100 allelic variants are described [35]. Genetic polymorphisms may result in non-functional or reduced function alleles. Copy number variations, such as *CYP2D6* gene deletions and *CYP2D6* gene duplications, also occur [31][36]. This genetic variability results in individuals showing a broad spectrum of enzyme activities, indicated as poor (PM), intermediate (IM), normal (NM) or ultra-rapid metabolizers (UM) based on genetic composition [17][36]. Another approach is to use Activity Scores (AS), in which normal alleles are assigned a value of 1.0, decreased activity alleles are assigned a score of 0.5, and null alleles are assigned a score of 0.0 [17]. Genetic variants can be specific for certain populations and rarely found in other populations [33][37]. The variation in allele frequency results in differences in metabolic CYP2D6 activity amongst ethnic groups [33].

A translation of *CYP2D6* genotype into predicted CYP2D6 phenotype is shown in Table 1. Important changes were published in 2019, where it was internationally agreed to harmonize the *CYP2D6*1/*4* interpretation from an Extensive/Normal metabolizer phenotype (CPIC definition until 2017, mostly used in US) into an Intermediate Metabolizer phenotype (definition used by the Dutch Pharmacogenetics Working Group (DPWG), which is mostly used in Europe). The second important change concerns the *CYP2D6*10* allele, which was downgraded from AS = 0.5, comparable to other decreased activity alleles such as *9 and *41, to AS = 0.25 [38][39].

Table 1. Adapted final consensus *CYP2D6* genotype to phenotype table. Combining the previous CPIC and DPWG guidelines and adding new pharmacogenetic insights [38]. Abbreviations: CYP2D6: Cytochrome P450 2D6, UM: ultra-rapid metabolizer, NM: normal or extensive metabolizer, IM: intermediate metabolizer, PM: poor metabolizer, CPIC: Clinical Pharmacogenetic Implementation Consortium, DPWG: Dutch Pharmacogenetics Working Group.

Likely Phenotype	CURRENT CPIC Activity Score Definition	CURRENT DPWG Activity Score Definition	NEW Standardized Activity Score Definition
CYP2D6 UM	>2	>2.5	>2.25

CYP2D6 NM	1–2	1.5–2.5	1.25–2.25
CYP2D6 IM	0.5	0.5–1.0	0.25–1.0
CYP2D6 PM	0	0	0

Most recently, Lee et al. suggested a dichotomization into normal and slow metabolizer CYP2D6 groups in an effort to improve and simplify the current system [40]. Nonetheless, these authors also recommend considering the direct measurement of endoxifen plasma concentrations, as *CYP2D6* genotype is not solely responsible for systemic endoxifen levels. In line with this recommendation, several authors [41][42][43][44] as well as the CPIC tamoxifen guideline [17] suggest that individualized dosing (e.g., therapeutic drug monitoring, TDM) might be a better option, instead of using a standard dosage of 20 mg tamoxifen per day. Another option is direct phenotyping, using dextromethorphan as a CYP2D6 phenotyping probe [45][46][47]. The advantage of this approach is that no genotype to phenotype translation is required, and that dose adjustment can be determined before the start of therapy [47]. Nevertheless, there are many more metabolites with unknown or estrogen-like properties [48]. Therefore, tamoxifen dosing solely based on (predicted) endoxifen blood concentrations might not be the best approach.

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