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Induction of hepatic drug-metabolising enzymes and tamoxifen metabolite profile in relation to administration route during low-dose treatment in nude rats

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Abstract

Tamoxifen is the most used anticancer drug and is approved for chemoprevention. Little is known about the enzyme inducing properties of low-dose regimens and the influence of route of administration. In this study, nude rats received 5 mg/kg/day of tamoxifen orally or a 50 mg continuous-release pellet subcutaneously. The mRNAs for cytochrome P450-enzymes (CYPs), flavin-containing monooxygenase 1 (FMO1) and phase II drug-metabolising enzymes were quantified by real-time RT-PCR. Tamoxifen and metabolite concentrations were measured using HPLC. We observed a significant increase in CYP3A18 and FMO1 mRNA expression levels in the orally treated animals, whereas the increase in CYP3A2 expression did not reach statistical significance ($p = 0.057$). No significant induction of enzyme expression was observed in rats that received subcutaneous (S.c.) treatment. After 33 days the serum levels of 4-hydroxytamoxifen (4OHtam), tamoxifen and *N*-desmethyltamoxifen (NDtam) in orally treated animals were 1.8 ± 0.7, 11.1 ± 3.2 and 11.4 ± 3.8 ng/ml, respectively. In subcutaneously treated animals, tamoxifen and *N*-desmethyltamoxifen were detected in tissues, but not in serum. These data demonstrate that in contrast to the subcutaneous administration, low-dose oral tamoxifen induced tamoxifen-metabolising enzymes. Furthermore, the different routes of administration resulted in different serum and tissue levels of tamoxifen and metabolites.

Keywords: Tamoxifen; Cytochrome P450; CYP; Enzyme induction; Pharmacokinetics

1. Introduction

Tamoxifen is an antioestrogen that is widely used for the treatment of breast cancer and the only drug approved for the prevention of this disease [1]. It may act as a full oestrogen agonist, partial agonist or antagonist depending on the dose, animal species, sex, and target organ [2]. Its oestrogen agonistic properties cause side effects like endometrial cancer and thromboembolic diseases, which may limit its use in healthy women. While large trials have shown that tamoxifen significantly reduces breast cancer incidence in high-risk women, data on the overall safety of tamoxifen have shown mixed results [1,3–5].

The cytochrome P450 enzymes (CYPs) are hemoproteins that catalyze the metabolism of xenobiotics including tamoxifen (Fig. 1). CYP3A4, the major CYP3A isoform in humans, is abundantly present in the liver and small intestine [6,7]. The interindividual variation in the levels of CYP3A4 expression is reported to be up to 20-fold or more [6] and may account for the variability in the disposition of drugs metabolised by this enzyme [8]. The CYPs 2C9, 2D6 and 3A4 together account for 60–70% of all phase I metabolic biotransformations of drugs in humans [9].

In rats the four CYP3A-isoforms CYP3A1/3A23, 3A2, 3A9 and 3A18 have been identified. They are variably expressed depending on age, sex and inducer type [10], and

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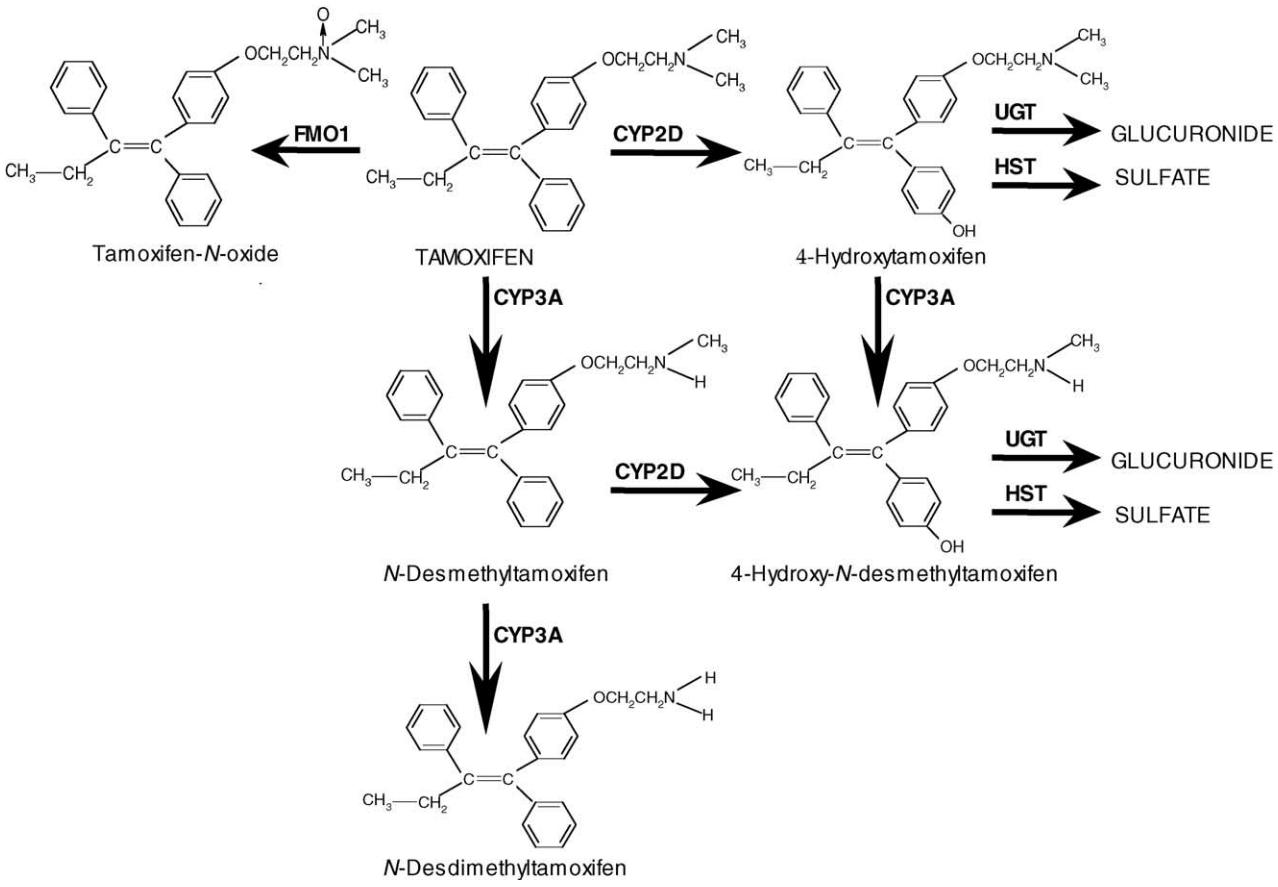

Fig. 1. Proposed metabolic pathways of tamoxifen in rat. CYP: cytochrome P450, FMO: flavin-containing monooxygenase, HST: hydroxysteroid sulfotransferase, UGT: UDP-glucuronosyltransferase.

seem to differ in tissue distribution, expression level, regulation, substrate specificity and catalytic activities. CYP3A1 and CYP3A23 have been demonstrated to be the same CYP3A isoform [11].

A wide variation in the activity of tamoxifen metabolising enzymes between individuals has been reported [12]. Tamoxifen is metabolised to the very potent metabolite 4-hydroxytamoxifen (4OHtam) and also to 4-hydroxy-*N*-desmethyltamoxifen (4OHNDtam) by CYP2D6 in humans and is demethylated mainly by CYP3A4. Tamoxifen has been shown to induce CYP3A expression in rats [13,14] and in human hepatocytes [15], whereas CYP2D6 has a polymorphic distribution that divide populations into "slow" or "rapid" metabolisers [16], and demonstrates similar substrate-specificity as rat CYP2D2 [17]. In vitro studies using recombinant human CYPs have also indicated a contribution of CYP2B6, 2C9, 2C19 and 3A4 in the hydroxylation process of tamoxifen [16,18,19], although the findings seem inconclusive [20].

Members of the CYP3A-family have been shown to catalyse the α-hydroxylation and *N*-demethylation of tamoxifen also in rats [21,22]. Human flavin-containing monooxygenase (FMO) and the main FMO isoform in rat liver, FMO1, demonstrate corresponding substrate-specificities, and are probably responsible for *N*-oxidation of tamoxifen [23]. Further metabolic processing of tamoxifen is performed by phase II enzymes, e.g. sulfation by hydroxysteroid sulfotransferases (HSTs) [24,25] and glucuronidation by UDP-glucuronosyltransferases (UGTs) [24].

We recently observed in a clinical study that a dose reduction of tamoxifen from the conventional 20 mg/day to 1 mg/day still resulted in full antagonistic activity on the expression of the cancer proliferation marker Ki-67 [26]. This observation is of interest since the side effects of tamoxifen may be concentration dependent [27]. An increased risk of endometrial cancer has been associated with duration of tamoxifen treatment and accumulated dose [28,29]. Furthermore, the activity and side effects of tamoxifen may be attributed not only to concentrations of the parent drug, but also to its biologically active metabolites and their accumulation in target tissues. Dose-finding studies and studies on the pharmacokinetics and pharmacodynamics of tamoxifen are therefore of high importance.

We have previously reported a change in the pharmacokinetics of tamoxifen in nude rats after 2–3 weeks of treatment with a substantial second increase in *N*-desmethyltamoxifen (NDtam) levels in serum after 2 weeks of treatment [30].