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Excretion of Hydroxylated Metabolites of Tamoxifen in Human Bile and Urine

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Abstract. *The selective oestrogen-receptor modulator tamoxifen is the most commonly used drug against breast cancer. It has potent metabolites, such as 4-hydroxytamoxifen. Recently, the metabolite 4-hydroxy-N-desmethyltamoxifen has received increased attention as it may be a major contributor to the overall effects of tamoxifen. The excretion of tamoxifen and its metabolites was examined in a patient with biliary drainage after an oral dose of [^{14}C]tamoxifen. During the first 10 days after oral dosing, 11.5, 26.7 and 24.7% of the radioactivity was excreted in the bile, urine and faeces, respectively. After deconjugation with beta-glucuronidase, the concentrations of tamoxifen and 4 of its metabolites were measured, and it was observed that the hydroxylated metabolites were excreted in the bile and urine. 4-Hydroxytamoxifen was the dominant compound, being detected during the first day of observation, whereas 4-hydroxy-N-desmethyltamoxifen was first observed in the urine and bile after 4 days. This is the first report on tamoxifen excretion in human bile and urine demonstrating that 4-hydroxytamoxifen may be a first-pass metabolite. In contrast, the potent metabolite 4-hydroxy-N-desmethyltamoxifen was first detected 4 days after administration of a single oral dose.*

Tamoxifen (tam) is a first generation selective oestrogen-receptor modulator (SERM), which is used as first- or second-line endocrine treatment of breast cancer and as a chemopreventive agent (1). Several cytochrome P450 (CYPs) enzymes are involved in the metabolism of tamoxifen, which is complex and involves, among others, N-demethylation and aromatic hydroxylation (Figure 1) (2-10). The metabolites 4-hydroxytamoxifen (4OHtam) and 4-hydroxy-N-desmethyltamoxifen (4OHNDtam) are products of the hepatic oxidation enzymes, CYP2D6 and CYP3A4, in humans. CYP2D6 hydroxylates tamoxifen and is polymorphic (2), whereas CYP3A4 demethylates tamoxifen and is inducible (11, 12). *In vitro* studies indicate a variable contribution of other CYPs in the hydroxylation process of tamoxifen (3, 4, 7).

The hydroxylated metabolites of tamoxifen are more potent anti-oestrogens than the parent compound (13-16). Accordingly, 4OHNDtam, which has been named endoxifen (6), and 4OHtam contribute to the effects and side-effects of tamoxifen.

The aim of this study was to examine the excretion of tamoxifen and its metabolites in a patient with bile duct drainage. The patient gave written informed consent before participation in the study which was approved by the Regional Ethics Committee and the Norwegian Medicines Agency.

The data presented include analyses of complete samples of bile, urine and faeces during 10 days after a single oral dose of [^{14}C]-labelled tamoxifen. The results may contribute to an increased knowledge of the pharmacokinetics of this drug, whose metabolism includes first-pass metabolism, conversion to the potent hydroxylated metabolites and enterohepatic circulation. Recently, the metabolite 4OHNDtam has received increased attention as it may make a major contribution to the overall effects of tamoxifen (6, 17, 18).

Case Report

The patient was a 61-year-old female with a pancreatic head tumour. She weighed 57 kg. Due to occlusion of the common bile duct, a T-drain biliary drainage was established. One day later, she fasted overnight before receiving a single oral dose of 90.23 mg tamoxifen, as a capsule containing 219 μCi [^{14}C] tamoxifen.

The patient had a plasma creatinine level of 44-60 μmol/L (normal 60-120 μmol/L) and elevated serum bilirubin levels

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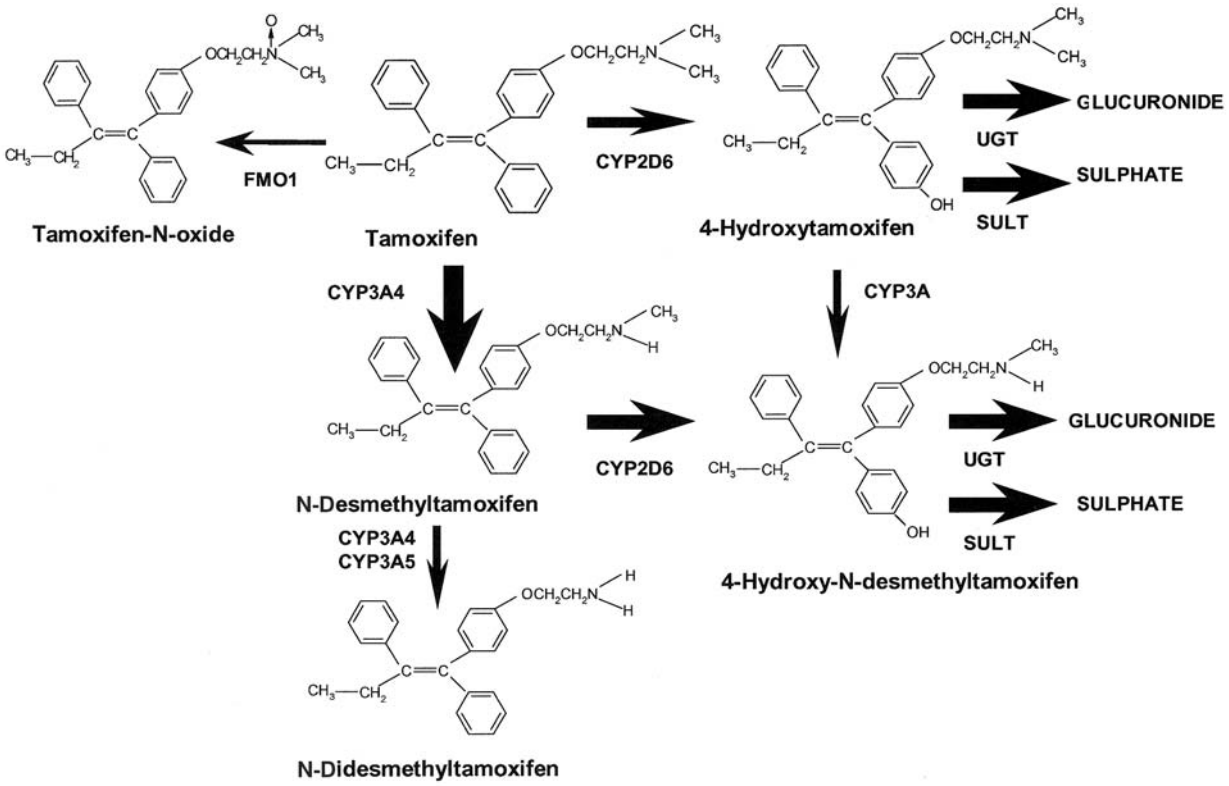

Figure 1. *Proposed major metabolic pathways of tamoxifen in humans. CYP, cytochrome P450; FMO, flavin-containing monooxygenase; SULT, sulphotransferase; UGT, UDP-glucuronosyltransferase.*

of 300-92 μmol/L (normal <17 μmol/L) before and during the 10-day sampling period. The gamma-glutamyltransferase levels were 895-496 U/L (normal <50 U/L), while the alanin aminotransferase levels before tamoxifen administration were 788 U/L (normal <50 U/L).

Thirteen days after tamoxifen administration, the pancreas was removed by a Whipple operation and the patient was discharged 2 weeks later.

Materials and Methods

[^{14}C]Tamoxifen citrate was donated by Imperial Chemical Industries Pharmaceuticals (Macclesfield, UK). The compound had a specific activity of 2.43 μCi/mg (1.37 mCi/mmol). The radiochemical purity of the compound was above 98%, as determined by thin-layer chromatography and high-pressure liquid chromatography (HPLC).

Bile, urine and faeces samples were collected for 10 days following tamoxifen administration. Blood samples were collected into vacutainers. The total volumes of urine, bile and faeces were collected into tight containers and frozen immediately at –20˚C. All HPLC analyses were performed within 30 days after sampling. The patient did not receive any systemic anticancer therapy other than the medication used in this study.

Treatment of samples from the urine, bile and faeces with beta-glucuronidase was performed as described previously (19). The concentrations of tamoxifen and its metabolites were measured using an HPLC method with post-column, online photocyclization to phenanthrenes and fluorimetric detection (20).

The total radioactivity was measured using the Tri-Card liquid scintillation spectrophotometer, model 2100TR Packard Bioscience, (Groningen, NL). Samples were counted in Opti-Fluor cocktail (Packard Bioscience). All counts were corrected for quench and converted to disintegration/min (dpm) by a channel ratio procedure. The percentage dose excreted in the bile, urine or faeces was calculated as the sample dpm divided by the total dpm dosed to the subject x100. The elimination and absorption rate constants of tamoxifen were calculated from the residuals method (21).

Results and Discussion

The presence of a biliary T-drain represented an outstanding opportunity for the evaluation of the first-pass metabolism of tamoxifen. A total of 6896 ml of bile was obtained through the T-drain during the 10-day sampling period. A significant amount of tamoxifen and its metabolites contained in the bile was withdrawn from the enterohepatic circulation, thus shortening the half-life of the drug. On the other hand, the perturbed liver metabolism of the patient may have prolonged the half-life of tamoxifen. However, a terminal elimination phase with a half-life of tamoxifen in the serum of 5 days was observed, which is in keeping with the 5-7 days

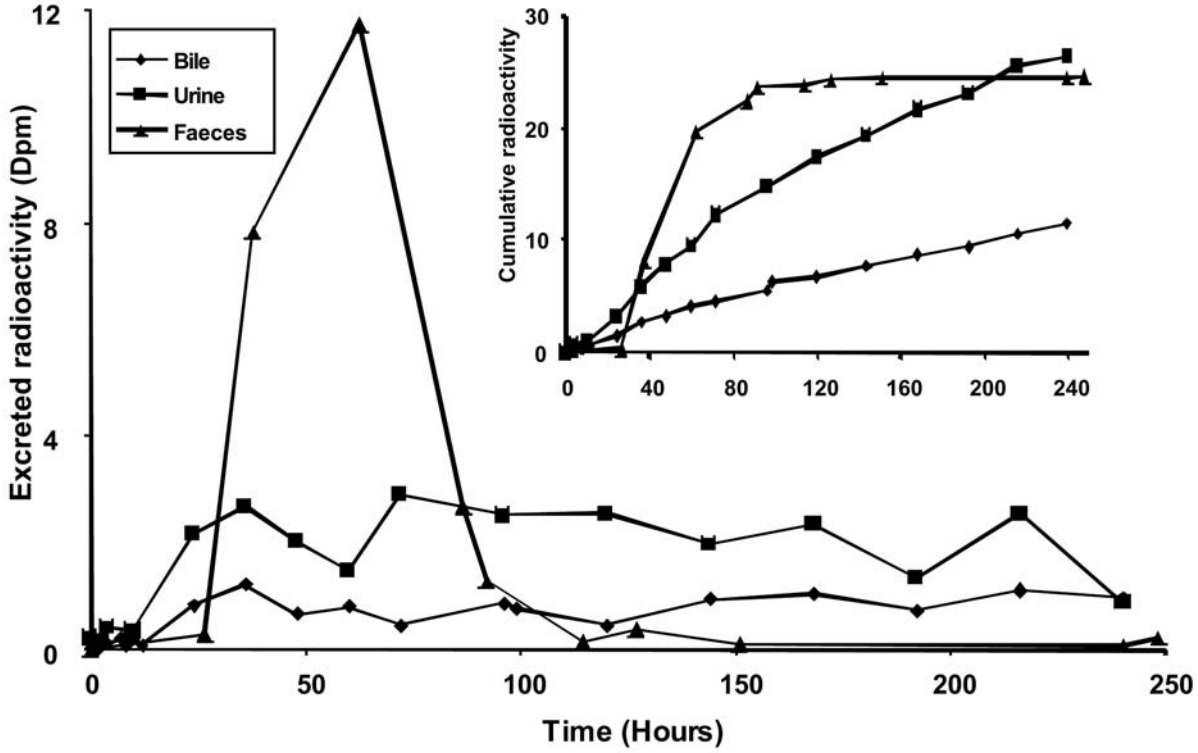

Figure 2. *Excreted radioactivity (dpm) in the faeces, urine and bile during 10 days as per cent of the administered dose. The insert shows the cumulative excretion.*

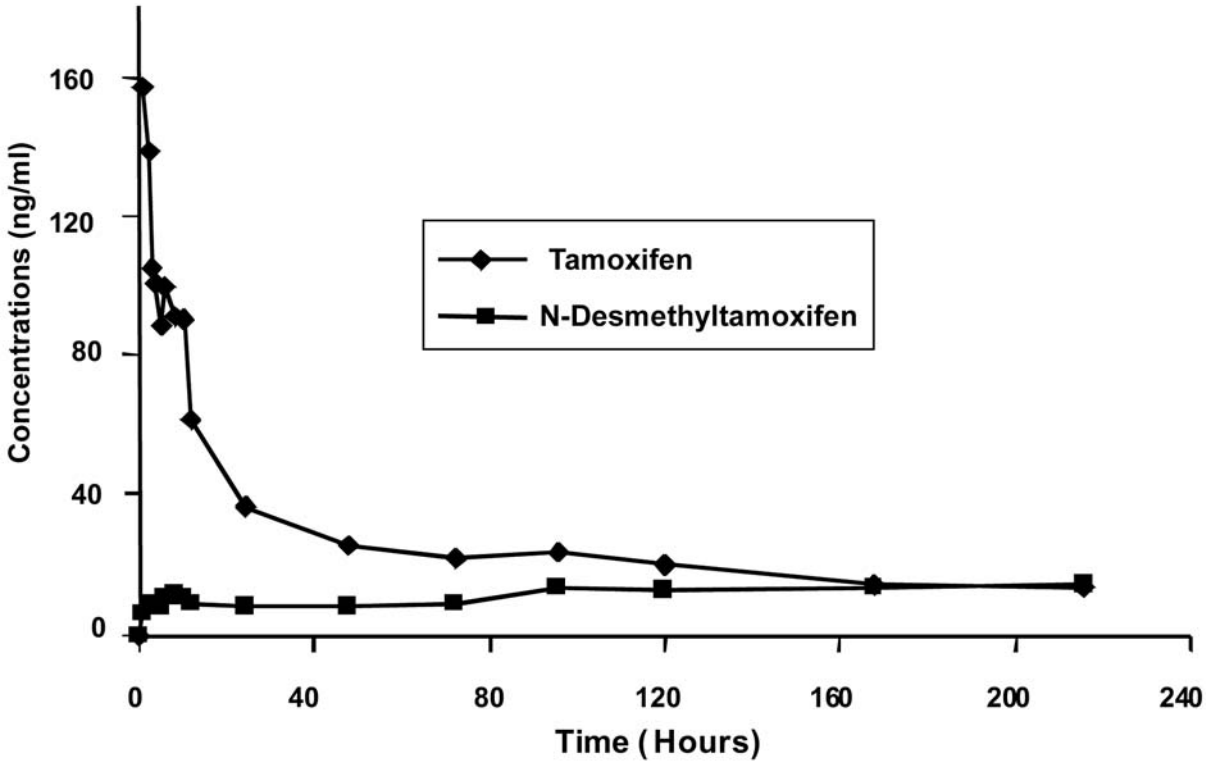

Figure 3. *Tamoxifen and metabolite concentrations in serum over a 10-day sampling period after a single oral dose of 90.23 mg [^{14}C] tamoxifen. HPLC analysis was performed after deconjugation with beta-glucuronidase.*

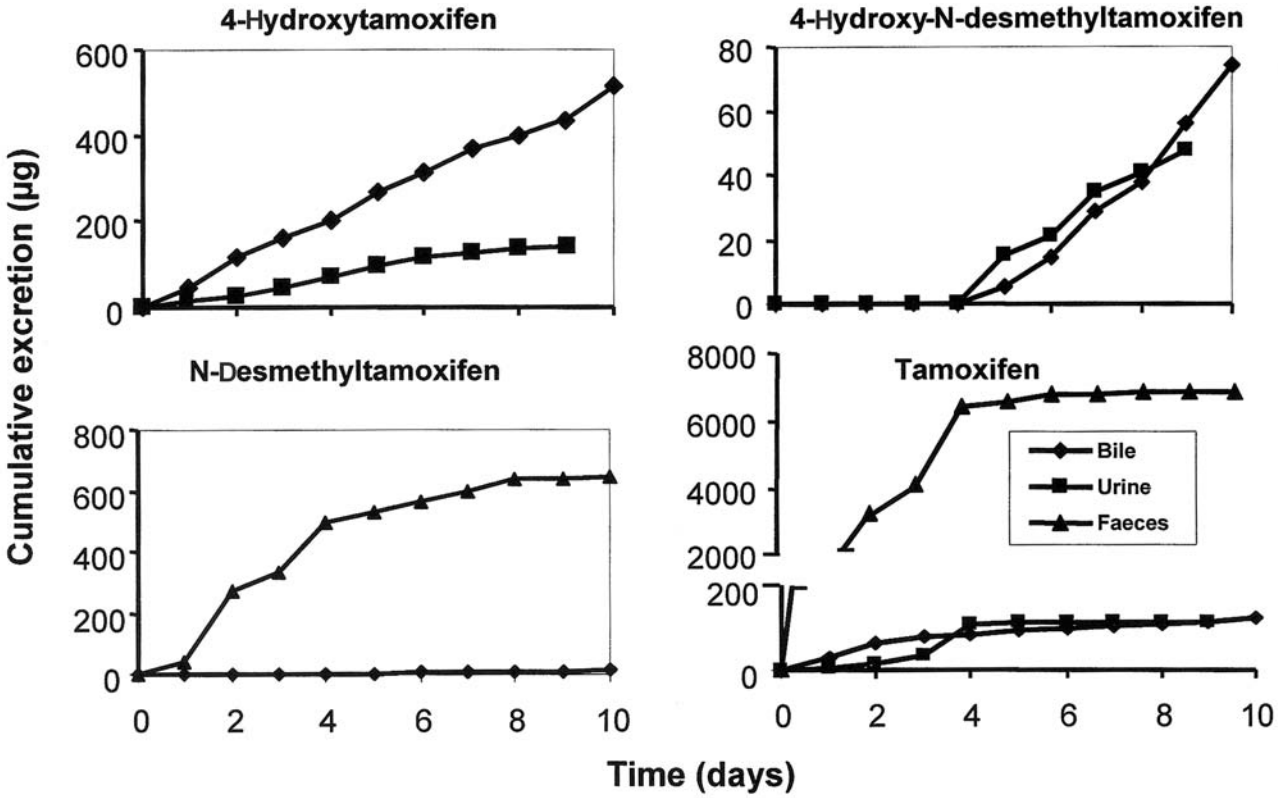

Figure 4. *Cumulative excretion of tamoxifen and its metabolites in the bile, urine and faeces after a single oral dose of 90.23 mg [^{14}C] tamoxifen (Y-axis scale is different between panels).*

observed by others (22-25). Furthermore, the recovery of 63% of the total radioactivity compares well with the results of Fromson *et al*. (26), who recovered 65% of the labelled dose 2 weeks after administration.

The cumulative excretion of total radioactivity linearly increased in the bile and urine over the study period, whereas it levelled-out in the faeces after 4 days (Figure 2). The portions of the administered dose recovered in the bile, urine and faeces during the sampling period were 11.5, 26.7 and 24.7%, respectively (Figure 2). This contrasts with the total amount of approximately 9.0 mg of tamoxifen and its metabolites detected by our HPLC method. Our data demonstrated that the major products of tamoxifen were not detected by our HPLC analysis performed after deconjugation with beta-glucuronidase. Sulphate conjugates of tamoxifen and its metabolites may represent such products. The sulphotransferase 1A1, which in part is responsible for elimination of 4OHtam, has recently been linked to the efficacy of the drug (27, 28).

Only tamoxifen and N-desmethyltamoxifen (NDtam) levels were observed in the serum (Figure 3). The occurrence of a peak concentration of tamoxifen in the serum within 1 hour of administration suggests a good and fast absorption of the drug. After an initial rapid increase, NDtam increased slowly during the observation period. Based on the serum concentrations measured, the absorption half-life of tamoxifen was calculated as 5 hours. In serum, the hydroxylated metabolites 4OHtam and 4OHNDtam were below the detection limit of 1 ng/ml of the HPLC assay during the study period (Figure 3), suggesting that once formed, these metabolites are rapidly conjugated and eliminated in the bile and urine, resulting in undetectable serum levels (29).

In the bile and urine, 4OHtam was detected 2-4 hours after tamoxifen administration. In contrast, 4OHNDtam was first detected after 4 days (Figure 4). 4OHtam represented the largest fraction excreted in the bile, while 4OHtam and 4OHNDtam were the only metabolites detected in the bile and urine. The metabolic pathway to 4OHtam involves only one metabolic step, whereas that of 4OHNDtam includes N-demethylation as well as hydroxylation. The 4-day lag-time prior to excretion in the bile and urine suggests that the demethylating step is rate limiting. Furthermore, the findings indicate that 4OHtam is a first-pass metabolite, as opposed to 4OHNDtam that was first observed in the bile and urine 4 days after

tamoxifen had entered the central compartment. A slower conjugation and excretion of 4OHNDtam compared to 4OHtam may explain why NDtam and 4OHNDtam were present in higher concentrations in the serum during chronic tamoxifen treatment.

In the faeces, tamoxifen was the largest fraction excreted, representing almost 8 mg (Figure 4). This is in agreement with previous observations, indicating that the major route of excretion (in human and in laboratory animals investigated) is *via* the faeces (26, 30). The hydroxylated metabolites 4OHtam and 4OHNDtam were not detected in the faeces and a peak concentration of tamoxifen was observed 62 hours after tamoxifen administration. Tamoxifen and NDtam then declined sharply to reach levels below 5% of the peak concentration after 94 hours. As the major amount of tamoxifen excreted was observed during the first few days, this may represent mainly the unabsorbed drug rather than the excreted drug. Interestingly, the demethylating enzyme CYP3A4 and the multidrug efflux pump P-glycoprotein are present at high levels in the enterocytes in the gastrointestinal tract. Accordingly, the NDtam observed in the faeces may originate from tamoxifen which has been demethylated in the enterocytes and back-transported by P-glycoprotein into the intestinal lumen (31).

In conclusion, a fast absorption of tamoxifen was observed after oral dosing. Overall, 63% of the [^{14}C] radioactivity administered was excreted during the following 10 days. The amounts recovered in the bile, urine and faeces were 11.5, 26.7 and 24.7%, respectively. The elimination of tamoxifen in this patient with a moderate liver disease and T-drain biliary drainage was comparable to that observed in patients without liver insufficiency. After treatment of the bile and urine with beta-glucuronidase, 4OHtam was detected as the dominant compound. 4OHtam was excreted during the first day. In contrast, 4OHNDtam was first observed after 4 days and increased sharply thereafter. The results indicate that 4OHtam may be a main excretory product of tamoxifen.

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References

1 Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L and Wolmark N: Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst *90*: 1371-1388, 1998.

2 Dehal SS and Kupfer D: CYP2D6 catalyzes tamoxifen 4-hydroxylation in human liver. Cancer Res *57*: 3402-3406, 1997.

3 Crewe HK, Ellis SW, Lennard MS and Tucker GT: Variable contribution of cytochromes P450 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. Biochem Pharmacol *53*: 171-178, 1997.

4 Boocock DJ, Brown K, Gibbs AH, Sanchez E, Turteltaub KW and White IN: Identification of human CYP forms involved in the activation of tamoxifen and irreversible binding to DNA. Carcinogenesis *23*: 1897-1901, 2002.

5 Sridar C, Kent UM, Notley LM, Gillam EM and Hollenberg PF: Effect of tamoxifen on the enzymatic activity of human cytochrome CYP2B6. J Pharmacol Exp Ther *301*: 945-952, 2002.

6 Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P, Hayes DF, Desta Z and Flockhart DA: Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst *95*: 1758-1764, 2003.

7 Coller JK, Krebsfaenger N, Klein K, Wolbold R, Nussler A, Neuhaus P, Zanger UM, Eichelbaum M and Murdter TE: Large interindividual variability in the *in vitro* formation of tamoxifen metabolites related to the development of genotoxicity. Br J Clin Pharmacol *57*: 105-111, 2004.

8 Desta Z, Ward BA, Soukhova NV and Flockhart DA: Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system *in vitro*: prominent roles for CYP3A and CYP2D6. J Pharmacol Exp Ther *310*: 1062-1075, 2004.

9 Mani C, Hodgson E and Kupfer D: Metabolism of the antimammary cancer antiestrogenic agent tamoxifen .2. Flavin-containing monooxygenase-mediated N-oxidation. Drug Metab Dispos *21*: 657-661, 1993.

10 Guengerich FP: Cytochrome P-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol *39*: 1-17, 1999.

11 Mani C, Pearce R, Parkinson A and Kupfer D: Involvement of cytochrome P4503A in catalysis of tamoxifen activation and covalent binding to rat and human liver microsomes. Carcinogenesis *15*: 2715-2720, 1994.

12 Cotreau MM, von Moltke LL, Harmatz JS and Greenblatt DJ: Molecular and pharmacokinetic evaluation of rat hepatic and gastrointestinal cytochrome p450 induction by tamoxifen. Pharmacology *63*: 210-219, 2001.

13 Furr BJA and Jordan VC: The pharmacology and clinical uses of tamoxifen. Pharmac Ther *25*: 127-205, 1984.

14 Fabian C, Tilzer L and Sternson L: Comparative binding affinities of tamoxifen, 4-hydroxytamoxifen, and desmethyltamoxifen for estrogen receptors isolated from human breast carcinoma: correlation with blood levels in patients with metastatic breast cancer. Biopharm Drug Dispos *2*: 381-390, 1981.

15 Robertson DW, Katzenellenbogen JA, Hayes JR and Katzenellenbogen BS: Antiestrogen basicity-activity relationships: a comparison of the estrogen receptor binding and antiuterotrophic potencies of several analogues of (Z)-1,2-diphenyl-1-[4-[2-(dimethylamino)ethoxy]phenyl]-1-butene (tamoxifen, nolvadex) having altered basicity. J Med Chem *25*: 167-171, 1982.

16 Katzenellenbogen BS, Norman MJ, Eckert RL, Peltz SW and Mangel WF: Bioactivities, estrogen receptor interactions, and plasminogen activator-inducing activities of tamoxifen and hydroxytamoxifen isomers in MCF-7 human breast cancer cells. Cancer Res *44*: 112-119, 1984.

17 Johnson MD, Zuo H, Lee KH, Trebley JP, Rae JM, Weatherman RV, Desta Z, Flockhart DA and Skaar TC: Pharmacological characterization of 4-hydroxy- N -desmethyl tamoxifen, a novel active metabolite of tamoxifen. Breast Cancer Res Treat *85*: 151-159, 2004.

18 Lim YC, Desta Z, Flockhart DA and Skaar TC: Endoxifen (4-hydroxy-N-desmethyl-tamoxifen) has anti-estrogenic effects in breast cancer cells with potency similar to 4-hydroxy-tamoxifen. Cancer Chemother Pharmacol [In Press], 2005.

19 Lien EA, Solheim E, Lea OA, Lundgren S, Kvinnsland S and Ueland PM: Distribution of 4-hydroxy-N-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res *49*: 2175-2183, 1989.

20 Lien EA, Ueland PM, Solheim E and Kvinnsland S: Determination of tamoxifen and four metabolites in serum by low-dispersion liquid chromatography. Clin Chem *33*: 1608-1614, 1987.

21 Rowland M and Tozer TN: Clinical Pharmacokinetics: Concepts and Applications, Third edition, Philadelphia, PA, Lea & Febiger, pp. 478, 1995.

22 Patterson JS, Settatree RS, Adam HK and Kemp JV: Serum concentrations of tamoxifen and major metabolite during long term nolvadex therapy, correlated with clinical response. *In:* Breast Cancer – Experimental and Clinical Aspects. Mouridsen HT and Palshof T (eds.). Oxford, Pergamon Press, pp. 89-92, 1980.

23 Tukker JJ, Blankenstein MA and Nortier JWR: Comparison of bioavailability in man of tamoxifen after oral and rectal administration. J Pharm Pharmacol *38*: 888-892, 1986.

24 de Vos D, Guelen PJM and Stevenson D: The biovailability of tamoxifen: new findings and their clinical implications. Curr Ther Res *46*: 703-708, 1989.

25 Herrlinger C, Braunfels M, Fink E, Kinzig M, Metz R, Sørgel F and Vergin H: Pharmacokinetics and bioavailability of tamoxifen in healthy volunteers. Int J Clin Pharm Ther Toxicol *30*: 487-489, 1992.

26 Fromson JM, Pearson S and Bramah S: The metabolism of tamoxifen. (I.C.I. 46, 474) Part II: In female patients. Xenobiotica *3*: 711-714, 1973.

27 Nowell S, Sweeney C, Winters M, Stone A, Lang NP, Hutchins LF, Kadlubar FF and Ambrosone CB: Association between sulfotransferase 1A1 genotype and survival of breast cancer patients receiving tamoxifen therapy. J Natl Cancer Inst *94*: 1635-1640, 2002.

28 Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH, Skaar T, Storniolo AM, Li L, Araba A, Blanchard R, Nguyen A, Ullmer L, Hayden J, Lemler S, Weinshilboum RM, Rae JM, Hayes DF and Flockhart DA: CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst *97*: 30-39, 2005.

29 Adam HK, Patterson JS and Kemp JV: Studies on the metabolism and pharmacokinetics of tamoxifen in normal volunteers. Cancer Treat Rep *64*: 761-764, 1980.

30 Adam HK: A review of the pharmacokinetics and metabolism of "Nolvadex" (tamoxifen). *In:* Non-steroidal Antioestrogens: Molecular Pharmacology and Antitumour Activity. Sutherland RL and Craig-Jordan V (eds.). London, Academic Press, pp. 59-74, 1981.

31 Zhang Y and Benet LZ: The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein. Clin Pharmacokinet *40*: 159-168, 2001.

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