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INFLUENCE OF TAMOXIFEN AND ITS *N*-DESMETHYL AND 4-HYDROXY METABOLITES ON RAT LIVER MICROSOMAL ENZYMES

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Abstract—Tamoxifen (Nolvadex; TAM) and its major metabolites, *N*-desmethyl- (DMT) and 4-hydroxy-tamoxifen (HT), were shown to be potent inhibitors of hepatic cytochrome P-450-dependent mixed function oxidations. From *in vitro* experiments, all three were found to be potent inhibitors of oxidation of Type-I substrates (ethylmorphine and aminopyrine) and less potent, non-competitive inhibitors of Type-II substrates (aniline and dimethylnitrosamine). TAM, DMT and HT were of essentially equal potency and had a much more pronounced effect on Type-I substrates than on Type-II compounds studied. Their action appears to parallel SKF-525A in type and potency of inhibition produced. Spectral binding studies suggest that TAM and its metabolites exert their effects by occupying the Type-I binding site of cytochrome P-450 and thus limiting the accessibility of other substrates to the active site of the enzyme. TAM (and its metabolites) also inhibits its own metabolism, altering the distribution and elimination half-lives of tamoxifen-derived species. In addition, tamoxifen metabolism was found to be sensitive to the presence of other drugs. These results raise concern regarding the role that continued administration of tamoxifen plays in changing its own disposition as well as in the detoxification of drugs administered with it.

Tamoxifen (Nolvadex; TAM) is a non-steroidal (i.e. substituted phenylstilbene) anti-estrogen that is used clinically in the management of metastatic breast cancer and as an adjunct to surgical ablation of the primary tumor in estrogen receptor positive (primarily post-menopausal) patients [1–5]. The drug is metabolized extensively by cytochrome P-450-dependent hepatic mixed function oxidases in man [6] and various other mammalian species [7, 8], yielding primarily the *N*-desmethyl (DMT) and 4-hydroxy (HT) metabolites. In man, at steady state, DMT levels are 1 to 1.5 times greater than TAM levels, and HT concentrations are *ca* 5% of TAM levels [9]. TAM and its metabolites all exhibit long elimination half-lives ($\geq$ 7 days) from blood following discontinuation of use [9, 10]. These metabolites may, together with the parent, contribute to the observed clinical response since HT appears to be strongly anti-estrogenic (binding to estrogen receptors isolated from human breast carcinoma 50–75 times more strongly than TAM itself), while DMT is weakly estrogenic [11–13]. HT has also been bound to nuclear fraction of rat uterus following administration of [^{3}H]TAM [14, 15]. Thus, situations that change the relative distribution of tamoxifen species may influence the therapeutic effectiveness of the drug.

In an early pharmacokinetic study [16] single oral doses of TAM were administered to healthy male volunteers followed by a 28-day rest preceding a second challenge with drug. The elimination half-life of the parent drug increased with successive doses. A similar result was observed by Borgna and Rochefort [14]. Daily doses of unlabeled TAM administered prior to dosing with [^{3}H]TAM resulted in a decrease in the proportions of HT and DMT found in rat plasma relative to the levels found in the absence of TAM pretreatment. These results suggest the possibility that TAM (or its metabolites) may inhibit its own metabolism.

Al-Turk *et al.* [17, 18] have found that administration of TAM at a daily dose of 10 mg/kg results in a decrease in 7-ethoxycoumarin *O*-deethylase and aryl hydrocarbon hydroxylase (AHH) activities in male rats; however, in female rats, no effect on this deethylase was observed. It is the aim of this study to gain a clearer picture of the changes in metabolism produced by and occurring in TAM when exposed to secondary agents. Specifically, the effects of pretreatment of animals with the classical inducers of microsomal enzymes (a) phenobarbital or (b) 3-methylcholanthrene or with (c) TAM, (d) DMT or (e) HT on *in vitro* metabolism of TAM, ethylmorphine and aniline were studied; and in *in vitro* systems, the influence of TAM, DMT or HT on the metabolism of ethylmorphine, aminopyrine, aniline, *N*-nitrosodimethylamine and tamoxifen itself was also quantitatively defined. The first two compounds are classified as Type I substrates while aniline is a Type II substrate, classification being based on the difference spectrum produced by interaction of substrate with cytochrome P-450 which, in turn, is apparently related to specific sites of interaction with the coenzyme [19].

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EXPERIMENTAL

Chemicals. All biochemicals used in these studies, as well as 3-methylcholanthrene (3-MC), were purchased from the Sigma Chemical Co., St. Louis, MO. Aniline hydrochloride (recrystallized prior to use) and *p*-aminophenol were purchased from the Aldrich Chemical Co., Milwaukee, WI. Aminopyrine and sodium phenobarbital were obtained from Merck & Co., Rahway, NJ. TAM citrate, DMT and HT were provided by ICI Americas, Wilmington, DE, and ICI, Ltd., Macclesfield, England.

Treatment schedules. Female Sprague–Dawley rats (200–250 g), purchased from Sasco, Omaha, NE, were maintained on water and lab chow *ad lib.* When appropriate, animals were pretreated by daily intraperitoneal injection with either normal saline or corn oil (serving as controls) or with phenobarbital dissolved in saline (40 mg/kg × 3 days) or 3-MC (20 mg/kg × 5 days), TAM (31 mg/kg × 3 days), DMT (30 mg/kg × 3 days) or HT (32 mg/kg × 3 days) dissolved in corn oil. Animals were killed 24 hr after the last injection by cardiac puncture. Blood samples were obtained at time of death and livers were excised.

Binding studies. Absorbance spectra resulting from the binding of ethylmorphine and aniline to cytochrome P-450 were recorded using an American Instruments DW-2 spectrophotometer operated in the split beam mode. Ethylmorphine added to washed hepatic microsomes produces a typical Type I difference spectrum with λ_{max} at 385 nm and λ_{min} at 420 nm, whereas aniline produces a typical Type II difference spectrum with λ_{max} at 431 nm and λ_{min} at 390 nm. Using the dual wavelength mode, the absorbance differences (ΔA $\lambda_{max} - \Delta A$ λ_{min}) for ethylmorphine and aniline were measured at six concentrations ranging from 10^{-4} to 10^{-3} M [20] in the presence of TAM citrate (10^{-5} M to 3.3×10^{-4} M) dissolved in acetone–water (90:10).

Control experiments were carried out by determining the spectral change that occurs with the addition of ethylmorphine or aniline to microsomal suspensions containing the solvent [i.e. acetone–water (90:10) in the absence of TAM]. Spectral constants (K_s and ΔA_{max}) were calculated by the method of Wilkinson [21].

Metabolic incubation mixtures. The standard incubation mixture contained (9000 *g*) supernatant fraction equivalent to 1.75 g of liver (7 ml), phosphate buffer (400 μmoles; pH 7.4), semicarbazide (75.0 μmoles), $MgCl_2 \cdot 6H_2O$ (20 μmoles) and a cofactor consisting of NADP (4 μmoles), glucose-6-phosphate (40 μmoles) and glucose-6-phosphate dehydrogenase (6 I.U.) in a total volume of 10 ml. Incubation mixtures utilizing microsomes (1 ml of suspension equivalent to 250 mg of liver) were prepared with the same concentration of components as above; however, the final total volume was 5 ml. Microsomal protein concentration was determined by the method of Lowry *et al.* [22], and cytochrome P-450 was measured by difference spectroscopy according to the method of Omura and Sato [23]. Stock solutions of the substrates ethylmorphine (final concentration in incubation mixtures = 0.2 to 1.0 mM), aminopyrine (final concentration in incubation mixtures = 0.5 to 10 mM) and aniline (final concentration in incubation mixtures = 0.1 to 0.8 mM) were prepared in 1.15% KCl solution, while TAM, DMT and HT (final concentration in incubation mixtures = 0.02 to 1 mM) stock solutions were prepared in ethanol–acetone (1:1) solution. In all cases, the desired amounts of TAM and metabolic products were delivered in 25 μl of solvent. *N*-Nitrosodimethylamine (0.12 to 4 mM and 10 to 80 mM) was used to observe *N*-demethylase I and II activity respectively [24]. Mixtures were shaken at 120 cycles/min for 15 min at 37°. Control incubations were carried out using either 1.15% KCl or acetone–ethanol solvent as "substrate".

Analysis of incubation mixtures. Substrate demethylation was determined by measuring formaldehyde evolution [25]. Aniline hydroxylation was measured colorimetrically as the amount of *p*-aminophenol formed [26]. Levels of TAM, DMT and HT were determined in whole liver tissue (after appropriate digestion), liver homogenates and whole blood by high performance liquid chromatography (HPLC) following precolumn photochemical derivatization [27, 28]. Separations were carried out on a μBondapak C-18 column (10 μm particles; 300 × 4.6 mm; Waters Associates, Milford, MA) using a mobile phase of 60:40 acetonitrile:ammonium phosphate buffer (4.3 mM; pH 3.8) containing sodium octane sulfonate (1.9 mM). Column effluent was monitored fluorimetrically with the excitation monochromometer set at 256 nm, and emitted radiation passed through a 320 nm cut-off filter.

Data analysis. Enzyme kinetic constants were generated by fitting data by the Rosfit least squares method to a Lineweaver-Burk model [29]. This routine also provides indications of the type of inhibition produced.

RESULTS

Initial experiments were designed to determine the effect(s) of pretreatment of animals with "drugs" on the kinetics of metabolic oxidation of TAM in the 9000 *g* supernatant subfraction of liver homogenates obtained from female rats. Pretreatment of animals with known inducers of hepatic mixed function oxidases, phenobarbital and 3-methlcholanthrene, affected the kinetics of TAM N-demethylation and TAM hydroxylation to varying degrees (Table 1). 3-MC pretreatment modestly increased V_{max} and correspondingly increased K_m for TAM N-demethylation but more effectively stimulated TAM hydroxylation (V_{max} increasing by a factor of 2 with no significant change in K_m). Phenobarbital pretreatment increased K_m and V_{max} for TAM N-demethylation much more effectively than did 3-MC. It induced TAM hydroxylation to the same extent at 3-MC (expressed in terms of V_{max}) but with a large (~ 2-fold) increase in K_m (unlike that observed following 3-MC pretreatment).

A second group of animals was pretreated with either TAM, DMT or HT for 3 days prior to being killed. In these animals, the liver weight/body weight ratio, microsomal protein levels and cytochrome P-450 levels were all unchanged relative to the control group (i.e. animals pretreated with corn oil

Table 1. Effect of pretreatment of female rats for 3 days on hepatic-based metabolism

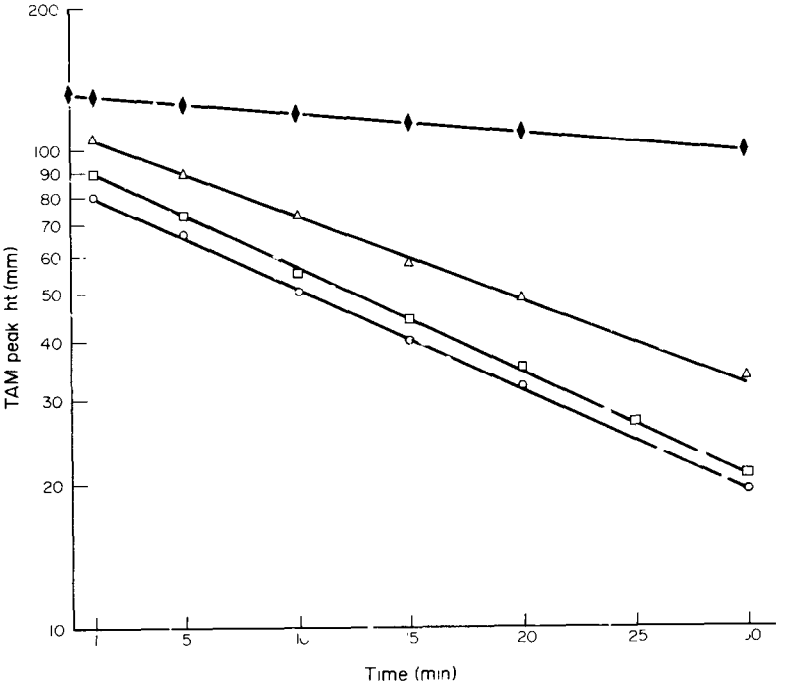
	Pretreatment*					
	Control†	Phenobarbital‡	3-MC‡	TAM‡	DMT‡	HT‡
Liver wt/body wt	0.035 ± 0.004	0.044 ± 0.004	0.039 ± 0.002	0.033 ± 0.005	0.034 ± 0.006	0.034 ± 0.001
Microsomal protein (mg/g liver)	1.82 ± 0.25	14.32 ± 2.06	4.60 ± 0.85	1.74 ± 0.53	1.69 ± 0.41	1.59 ± 0.72
Cytochrome P-450 (nmoles/mg microsomal protein)	0.78 ± 0.01	1.40 ± 0.09	1.02 ± 0.19	0.76 ± 0.01	0.81 ± 0.05	0.75 ± 0.03
Ethylmorphine demethylation						
K_m (μM)	0.88 ± 0.04	0.74 ± 0.018	0.59 ± 0.11	§		
V_{max} (μmoles/mg protein/hr)	1.97 ± 0.62	6.97 ± 0.90	1.57 ± 0.36	§		
Aminopyrine demethylation						
K_m (mM)	1.95 ± 0.74			§	1.81 ± 0.46	1.75 ± 0.38
V_{max} (μmoles/mg protein/hr)	0.17 ± 0.02			§	0.08 ± 0.01	0.08 ± 0.01
Aniline hydroxylation						
K_m (mM)	0.28 ± 0.06	0.51 ± 0.09	0.87 ± 0.19	0.24 ± 0.07	0.26 ± 0.02	0.25 ± 0.04
V_{max} (μmoles/mg protein/hr)	0.10 ± 0.01	0.19 ± 0.08	0.17 ± 0.02	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.01
TAM demethylation						
K_m (μM)	4.73 ± 0.10	7.23 ± 1.16	5.36 ± 0.14			
V_{max} (nmoles/mg protein/hr)	1.51 ± 0.03	2.64 ± 0.03	1.89 ± 0.03			
TAM hydroxylation						
K_m (μM)	5.63 ± 1.50	13.77 ± 2.44	7.51 ± 0.06			
V_{max} (nmoles/mg protein/hr)	0.12 ± 0.02	0.24 ± 0.03	0.23 ± 0.020			

* Animals (four to six) were pretreated on daily basis by intraperitoneal injection. Each value is the average ± S.D. of three determinations on at least three animals.

† Normal saline solution or corn oil.

‡ Doses as indicated in the Experimental section.

§ No demethylation observed.



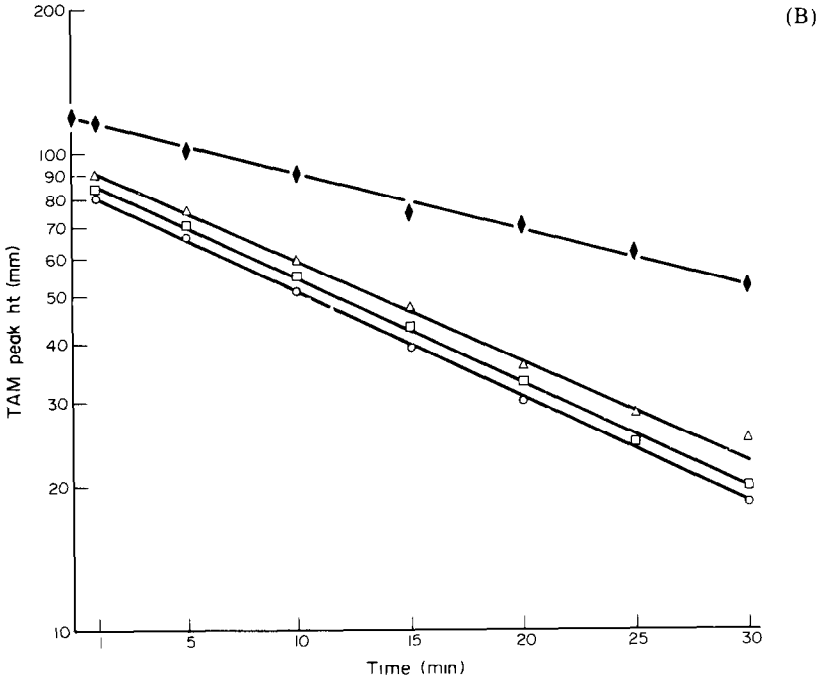


Fig. 1.(A) Rates of loss of TAM from liver microsomes from untreated rats in the presence of 0 μM (○), 5 μM (□), and 10 μM (△) DMT and from liver microsomes isolated from rats that had been pretreated with DMT, 30 mg/kg × 3 days (◆). (B) Kinetics of degradation of TAM from rat liver microsomes (isolated from untreated animals) in the presence of 0 μM (○), 5 μM (□), and 10 μM (△) HT and microsomes from rats treated with HT, 32 mg/kg × 3 days (◆).

Table 2. Levels of tamoxifen species following tamoxifen administration (31 mg/kg × 3 days, i.p.)

Compound	Tamoxifen concentration Liver (μg/g liver)	Blood (μg/ml)
TAM	1.99 ± 0.31	0.88 ± 0.04
DMT	6.37 ± 1.52	1.27 ± 0.07
HT	0.34 ± 0.02	0.16 ± 0.04

alone) (Table 1). In TAM-pretreated animals, accumulation of TAM, DMT and HT was noted in liver tissue (Table 2). DMT was present at 3 times the levels of parent drug in liver, while HT levels were *ca.* 17% of tissue levels of parent drug. The dose of TAM chosen for this pretreatment schedule was based on the original studies of Fromson *et al.* [7]. In TAM-pretreated animals, N-demethylation of ethylmorphine and aminopyrine was inhibited totally, while aniline hydroxylase activity was not altered significantly (Table 1). Both DMT and HT pretreatment inhibited aminopyrine N-demethlyation by *ca.* 50% but did not significantly inhibit aniline hydroxylase activity; in all cases no changes in K_m were observed relative to controls (Table 1).

The metabolism of TAM (measured in terms of loss of TAM) in the microsomal fraction was also inhibited by DMT and HT pretreatment. The loss of TAM in liver suspensions followed first order kinetic behavior with $k_{obs} = 0.05\ min^{-1}$ in control animals, while in HT-treated animals TAM degradation decreased by *ca* 40% ($k_{obs} = 0.03\ min^{-1}$) and in DMT-pretreated animals the rate of TAM loss was reduced by 80% ($k_{obs} = 0.01\ min^{-1}$) over controls (Fig. 1).

To define the role of TAM and its metabolites as mixed function oxidase inhibitors, a series of classical inhibition studies was carried out in which various concentrations of TAM, DMT or HT were incubated with suspensions of liver microsomes in the presence of various concentrations of other substrates (ethylmorphine, aminopyrine, aniline and *N*-nitrosodimethylamine) for the enzyme. Kinetics followed simple Michaelis-Menten behavior and could be analyzed from Lineweaver-Burk plots. Both DMT and HT are potent *competitive* inhibitors of ethylmorphine (Fig. 2) and aminopyrine demethylase. Inhibition constants (K_i) of 18.7 ± 6.2 μM and 16.4 ± 2.3 μM were calculated for inhibition of ethylmorphine demethylation DMT and HT, respectively, while K_i values of 7.5 ± 0.6 μM and 6.8 ± 1.2 μM were determined for inhibition of aminopyrine demethylation by DMT and HT respectively (Table 3). Both DMT and HT inhibited aniline hydroxylation but to a much lesser extent than was their effect on Type I substrates. Inhibition could only be observed at DMT or HT concentrations

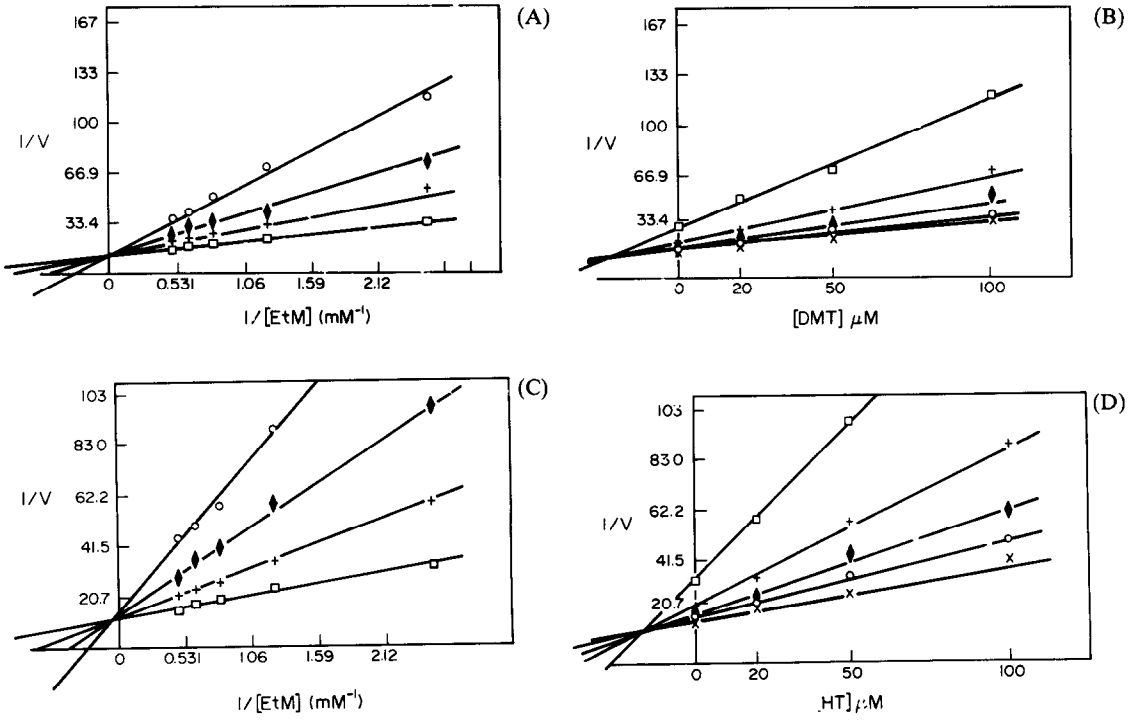


Fig. 2. (A) Lineweaver–Burk plot of ethylmorphine *N*-demethylase kinetics. EtM concentration ranged from 0.4 to 2.0 mM in the presence of 0 μM (□), 20 μm (+), 50 μM (◆) and 100 μM (○) DMT. (B) Dixon plot of ethylmorphine *N*-demethylase kinetics using 0.4 μM (□), 0.8 mM (+), 1.2 mM (◆), 1.6 mM (○) and 2.0 mM (×) EtM as substrate and DMT (20, 50 and 100 μM) as inhibitor. (C) Lineweaver–Burk plot of ethylmorphine *N*-demethylase kinetics. EtM concentration ranged from 0.4 to 2.0 mM in the presence of 0 μM (□), 20 μM (+), 50 μM (◆) and 100 μM (○) HT. (D) Dixon plot of ethylmorphine *N*-demethylase kinetics using 0.4 mM (□), 0.8 mM (+), 1.2 mM (◆), 1.6 mM (○) and 2.0 mM (+) EtM as substrate and HT (20, 50 and 100 μM) as inhibitor.

Table 3. Inhibition of microsomal oxidation by tamoxifen and its major metabolites

Enzyme system*	Inhibitor†	Inhibition type	K_i‡ (μM)
Ethylmorphine demethylase	TAM	Competitive	22.9 ± 2.3
	DMT	Competitive	18.7 ± 6.2
	HT	Competitive	16.4 ± 2.3
Aminopyrine demethylase	TAM	Competitive	20.0 ± 3.1
	DMT	Competitive	7.5 ± 0.6
	HT	Competitive	6.8 ± 1.2
Aniline hydroxylase	TAM	Non-competitive	184 ± 10
	DMT	Non-competitive	120 ± 5
	HT	Non-competitive	170 ± 2

* Studies carried out in microsomes are described in the Experimental section.
† Twenty-five microlitres of ethanol–acetone (1:1) solution containing 100, 250 or 500 nmoles of TAM, DMT or HT.
‡ Average ± S.D. for three determinations on each of three separate experiments.

$\geq 10^{-4}$ M, and fit a classical *non-competitive* (mixed) mechanism. K_i values of 120 ± 5 μM and 170 ± 2 μM were calculated for inhibition of aniline hydroxylation by DMT and HT respectively (Table 3).

The inhibitory effects of TAM on these substrate oxidations are less easily defined since TAM is itself a substrate for the enzyme system. It may be metabolized during the incubation, and inhibition would then be produced by a mixture of TAM, DMT and HT, where the reactive concentration of each was changing over the time of incubation, as represented schematically in Fig. 3. This scheme accounts for the metabolism of TAM as a competitive substrate as well as the inhibitory effects on secondary substrate oxidations produced by TAM itself as well as by DMT and HT. In the presence of ethylmorphine or aminopyrine (at the concentrations given in the Experimental section), oxidative metabolism of TAM (present at concentrations of 20–100 μM) was totally inhibited, as determined chromatographically. Aniline (0.1 to 0.8 mM) inhibited TAM biodegradation by >90%. Thus, *under the conditions*

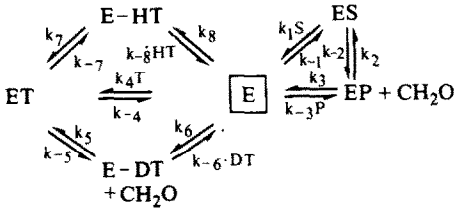


Fig. 3. Schematic representation of the interaction of Type I substrates and TAM (and its derived metabolites) with the cytochrome P-450-dependent mixed function oxidase system. Key: T = tamoxifen; DT = desmethyltamoxifen; HT = 4-hydroxytamoxifen; S = substrate for the enzyme; and P = product(s) of substrate metabolism.

of these experiments, TAM could be viewed as being metabolically inert and, therefore, any inhibition of oxidative metabolism observed in the presence of TAM would be due solely to TAM with no significant contribution from its metabolites. TAM was a potent *competitive* inhibitor of demethylation of the Type

Table 4. Influence of tamoxifen on *N*-nitrosodimethylamine (DMN) metabolism by rat liver microsomes

TAM concentration* (M)	K_m† (mM)	V_{max}† (nmoles/mg protein/hr)
	N-Demethylase I‡	
Control (acetone)	0.21 ± 0.08	51.4 ± 5.6
5×10^{-6}	0.29 ± 0.30	59.4 ± 15.6
5×10^{-5}	0.18 ± 0.05	50.8 ± 3.4
5×10^{-4}	0.18 ± 0.05	50.8 ± 3.4
	N-Demethylase II§	
Control (acetone)	35.00 ± 8.16	269.6 ± 30.9
5×10^{-6}	25.21 ± 4.75	245.6 ± 19.2
5×10^{-5}	17.02 ± 5.29	152.6 ± 16.8
5×10^{-4}	11.49 ± 6.11	74.8 ± 10.6

* Tamoxifen was dissolved in acetone and added at a constant volume to sample.
† Values represent the mean ± S.D. of three determinations on each of three separate experiments.
‡ Six substrate concentrations ranging from 0.125 to 4.0 mM DMN.
§ Six substrate concentrations ranging from 10.0 to 80.0 mM DMN.

Table 5. Influence of tamoxifen on binding spectra of ethylmorphine and aniline with cytochrome P-450*

Substrate	TAM concn.† (μM)	K_s‡ (mM)	ΔA_{max}‡ (ΔA/mg microsomal protein)
Ethylmorphine§	Control‖	0.033 ± 0.002	0.015 ± 0.001
	Control (acetone)¶	0.078 ± 0.009	0.015 ± 0.001
	10	0.012 ± 0.01	0.010 ± 0.001
	20	0.13 ± 0.02	0.009 ± 0.001
	50	0.15 ± 0.02	0.004 ± 0.001
	100	0.24 ± 0.04	0.001 ± 0.000
Aniline§	Control (acetone)¶	0.59 ± 0.08	0.016 ± 0.001
	50	0.64 ± 0.04	0.015 ± 0.001
	100	0.80 ± 0.11	0.015 ± 0.001
	330	1.04 ± 0.24	0.006 ± 0.001

* Experimental design as described in the text.

† TAM was added to cuvettes in a constant volume of acetone.

‡ Values represent the average ± S.D. for three determinations on each of three separate experiments.

§ Concentration range of substrate studies: 0.1 to 1.0 mM.

‖ Binding spectrum of substrate generated in the absence of TAM and any non-aqueous solvent.

¶ Binding spectrum of substrate generated in the absence of TAM, but with addition of the same amount of acetone used to deliver TAM to the system.

I substrates (Table 3), giving K_i values of 22.9 ± 2.3 μM and 20.0 ± 3.1 μM for ethylmorphine and aminopyrine demethylation respectively. TAM was a less effective inhibitor of the Type II substrate aniline, inhibition being demonstrated only at TAM concentrations $\geq 10^{-4}$ M. Inhibition was *non-competitive* (similar to the inhibition pattern of aniline hydroxylase by DMT and HT) and is quantitatively described by $K_i \sim 184 \pm 10$ μM (Table 3).

The inhibitory effects of TAM on *N*-nitrosodimethylamine demethylation were also determined. TAM (at concentrations as high as 500 μM) failed to inhibit the cytochrome P-450 independent *N*-demethylase I [29] (Table 4), i.e., no significant change in either K_m or V_{max}. However, at TAM concentrations of only 50 μM, *N*-demethylase II, a cytochrome P-450 dependent enzyme, [V_{max}] was *uncompetitively* inhibited by 40%; at 500 μM TAM, V_{max} was depressed by 71%.

Upon interaction with cytochrome P-450, many substrates produce difference spectra characteristics of the site and/or type of binding that takes place [18]. TAM itself produces a typical Type I difference spectrum λ_{max} = 390 nm and λ_{min} = 424 nm, suggesting that it interacts at the "Type I site". At concentrations of 10 and 100 μM, TAM inhibited the binding of ethylmorphine (another Type I substrate) to cytochrome P-450 by 35 and 94% respectively (Table 5). Aniline (a Type II substrate with spectral characteristics of λ_{max} = 431 nm and λ_{min} = 390 nm) binding to the cytochrome was also inhibited by TAM but to a lesser extent, i.e. minimal inhibition at a concentration $\leq 10^{-4}$ M with 60% inhibition at 3.3×10^{-4} M (Table 5). Carbon monoxide also serves as a ligand of cytochrome P-450 but it binds directly with the heme iron [19]. Its binding spectrum was not altered by TAM even at concentrations > 100 μM.

DISCUSSION

Tamoxifen is metabolized extensively by liver-based enzymes. In his original studies, Fromson *et al.* [6, 7] reported that in rats and in man the primary metabolite is HT, with lesser amounts of DMT being produced. Subsequently, it was determined that in man DMT [9, 30] is the major metabolite, with HT being produced in concentrations of *ca.* 5% of the parent drug [9, 16]. Several others have reported that DMT and HT appear in approximately equal amounts in rat plasma after [^{3}H]TAM administration [14, 15]. However, in our studies at a dose of 30 mg/kg of TAM, DMT was the primary plasma metabolite with lesser amounts of HT formed, suggesting it to be a reasonable animal model for studying TAM-biotransformation. This dose also resulted in accumulation of TAM and its DMT and HT metabolites in liver tissue as has been observed with other lipophilic drugs. The major species present in liver was DMT, suggesting that, following metabolism of TAM, the distribution characteristics of DMT, and to a lesser extent, HT, favor its retention by the tissue rather than further distribution and excretion. This hypothesis is supported by clinical pharmacokinetic data, indicating that TAM and DMT have long elimination half-lives [9, 10]. Robertson *et al.* [15] have also reported finding a hydroxy *N*-desmethyl metabolite of TAM, indicating that the metabolites are capable of undergoing further metabolic degradation. The persistence of TAM as well as its metabolic products in the liver suggests that the disposition of TAM itself as well as of other drugs (that may potentially be administered with it) may be modified, affecting both the efficacy and safety of these agents. The alteration in TAM metabolism observed following treatment with its metabolic products may provide an explanation for the

decrease in HT and DMT plasma concentrations when rats were first treated daily with unlabeled TAM and then with [^{3}H]TAM [14]. Subsequent experiments in which the *in vitro* metabolism of model substrates (representative Type I and Type II compounds) was studied in liver homogenates isolated from animals pretreated with TAM, DMT or HT confirmed the inhibitory activity of these species on microsomal oxidations. The absence of an effect on liver protein or cytochrome P-450 synthesis is consistent with the findings of Al-Turk *et al.* [17]; however, these compounds are potent inhibitors of N-dealkylation of the Type I substrates ethylmorphine and aminopyrine. In contrast, Al-Turk *et al.* [17] found that TAM failed to inhibit 7-ethyoxycoumarin O-deethylation in female rats, although appreciable inhibition was seen in male rats apparently due to alterations in testosterone levels. Through *in vitro* competitive substrate experiments, TAM, DMT and HT were shown to be essentially equipotent as inhibitors of mixed function oxidases. Here, too, the inhibitory effect was more pronounced toward Type I substrates occurring with potency of the same magnitude (expressed as K_i) as observed with SKF-525A [31]. Inhibition of Type I substrate oxidation was competitive (as is also seen with SKF-525A), suggesting that TAM is capable of occupying the same binding site as does ethylmorphine and aminopyrine (Type I substrates) and in this way inhibits oxidation. This hypothesis is supported by spectral binding studies of substrates to cytochrome P-450 which showed that TAM (a) produces a classical Type I spectrum with the cytochrome, and (b) competitively displaces ethylmorphine and aminopyrine from their binding sites. TAM species do not, however, bind directly with the iron of the cytochrome.

TAM, DMT and HT also inhibited the binding to cytochrome P-450 and the metabolism of aniline, a Type II substrate, but with much less effectiveness than was observed with the Type I substrates tested. Furthermore, TAM inhibited the cytochrome P-450-dependent *N*-demethylase (II) responsible for *N*-nitrosodimethylamine metabolism, but had no effect on the *N*-demethylase I system, which does not involve participation by cytochrome P-450. Inhibition of demethylase II was uncompetitive and was similar in type and magnitude to the inhibition of *N*-nitrosodimethylamine demethylation produced by SKF-525A [32]. All these data are consistent with the contention that TAM, DMT and HT are potent inhibitors of microsomal mixed function oxidase systems, acting primarily by interaction at or near the "Type I binding site" of cytochrome P-450.

These data suggest that, in the clinical situation where TAM is administered twice daily for prolonged periods (>2 years), it may interfere with the metabolism of other drugs that are co-administered with it.

In addition to demonstrating the effect of TAM on detoxification of various classical substrates, the metabolism of TAM itself was shown to be sensitive to the presence of other compounds. Pretreatment of animals with known inducers of microsomal oxidases, phenobarbital and 3-methylcholanthrene, resulted not only in the expected increase in metabolic activity, corresponding to increases in liver microsomal protein (per g liver) and cytochrome P-450 (and cytochrome P-448 in the case of 3-MC) levels, but also caused significant changes in the distribution of metabolites. The possible clinical consequences of such changes in product distribution have already been discussed.

Considering the wide range of medications routinely taken by cancer patients, concern must be raised regarding the possible role that TAM plays in their detoxication as well as in its own disposition. These concerns are intensified at a time [33] where TAM is being evaluated clinically in combination with potent cytotoxic agents (many of which exhibit small therapeutic indices). The relationship between metabolism of TAM and cytotoxic agents with which it is used is the subject of current investigation in our laboratory.

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