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SPECIES DIFFERENCES IN THE COVALENT BINDING OF [^{14}C]TAMOXIFEN TO LIVER MICROSOMES AND THE FORMS OF CYTOCHROME P450 INVOLVED

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Abstract—Species differences in the NADPH-dependent covalent binding of [^{14}C]tamoxifen to liver microsomes have been studied using preparations from humans, female F344 rats and DBA/2 mice. Protein binding has been used as an index of metabolic activation and as a surrogate for DNA binding in order to establish which forms of cytochrome P450 are responsible for genotoxicity. A panel of 12 human liver microsomes has been characterized and immunoquantified for nine cytochrome P450 isoenzymes. Binding of tamoxifen (45 μM) (25 ± 2.5 pmol/15 min/mg protein, mean ± SE) correlated ($P < 0.05$) with CYP3A4 and CYP2B6 content. Covalent binding of [^{14}C]tamoxifen to microsomal preparations from human breast tumour tissue could also be detected but at levels 7-fold lower than in liver. The covalent binding of tamoxifen to mice, rat or human liver microsomal preparations increased with increasing substrate concentration. Covalent binding of [^{14}C]tamoxifen (45 μM) in rats was 3.8-fold and mice 17-fold higher than in human liver microsomal preparations. In mice, the apparent K_m (9.6 ± 1.9 μM) was very much lower than for rats (119 ± 41 μM). Pretreatment of female rats with phenobarbitone or dexamethasone resulted in a 4- to 5-fold increase in [^{14}C]tamoxifen binding, relative to controls, consistent with the involvement of CYP2B1 and CYP3A1 in the metabolic activation. It cannot be distinguished at present if the same reactive metabolites are involved in protein and DNA binding. The greater potential of mouse liver microsomes to activate tamoxifen, relative to rats, does not reflect DNA damage or hepatocarcinogenicity seen following dosing with tamoxifen *in vivo*. It is concluded that covalent binding of tamoxifen to protein *in vitro* cannot be directly related to the carcinogenic potential of this compound. However, in the three species investigated, results suggest that the rat is a better model than the mouse for human liver microsomal activation of tamoxifen both with respect to kinetic parameters and the pattern of metabolic products.

Key words: tamoxifen; protein binding; metabolism; species; CYP-isoenzymes; induction

Tamoxifen, *Z*-(1-(4-(2-dimethylaminoethoxy)phenyl)-1,2-diphenyl-1-butene), is the main form of adjuvent therapy used in the treatment of breast cancer in women [1] and is currently undergoing clinical trials in women who are healthy but may be at risk from this disease [2]. For prophylactic use, a high degree of safety is required. Long-term administration to rats leads to the development of hepatocellular carcinomas [3–5], while mice appear to be resistant [6]. So far there is little evidence to suggest that exposure of women to this drug results in an increased incidence of liver tumours, although there are concerns that if the incidence of induced liver cancer was low, this would not be detected in a population of women undergoing treatment for breast cancer. There is also some evidence to suggest there may be an increased risk of tumours in other organs such as the endometrium [7, 8].

Administration of tamoxifen to rats or mice results in hepatic DNA damage which can be detected by ^{32}P-postlabelling [9, 10]. In order to exert such genotoxic effects, this drug has to undergo metabolic activation. A micronucleus assay using human lymphoblastoma (Crespi) cell lines expressing individual cytochrome P450 isoenzymes suggested that CYP3A4, CYP2E1 and possibly CYP2B6 were involved in the activation of tamoxifen to clastogenic intermediates [11]. All the *in vitro* tests for mutagenicity such as the Ames *Salmonella* assay have proved negative for this compound [10].

Following metabolic activation, tamoxifen binds covalently to microsomal protein. This reaction is catalysed by a NADPH and cytochrome P450-dependent reaction and is markedly increased by phenobarbital pretreatment [12]. The isoenzymic cytochrome P450 forms involved have not been studied. However, N-demethylation, a major detoxication route, is catalysed by the CYP3A family [13, 14], while in humans, CYP2C8 is associated with 4-hydroxylation [14]. In contrast, N-oxidation appears at least in part to be catalysed by a flavin-containing monooxygenase [15]. Binding of tamoxifen to exogenous DNA *in vitro* has not been directly demonstrated. However, DNA damage *in vitro*, using ^{32}P-postlabelling, has been shown. Rat

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and human microsomal incubation mixtures gave a similar level of ^{32}P-postlabelled adducts in both species [16].

The aim of the present study is to investigate the species specific activation of tamoxifen to a metabolite(s) which binds to microsomal protein. Covalent binding to protein has been used as a surrogate for DNA binding in order to identify the cytochrome P450 isoenzymic forms responsible. The potential of this drug to undergo metabolic activation is compared in rats and mice to see if this can be related to the susceptibility of the rodent species to liver cancer. If so, this might allow predictions to be made of potential human risk.

MATERIALS AND METHODS

Chemicals. Dexamethasone, β-naphthoflavone, tricaprylin, adenosine 2′-monophosphate and NADPH were from Sigma Chemical Co. Ltd. Tamoxifen was a gift from Dr J. Topham (Zeneca Pharmaceuticals, Macclesfield, Cheshire, U.K.). Ring [^{14}C]tamoxifen (sp. act. 56 mCi/mmol), of >98% radiochemical purity was from Cambridge Research Biochemicals (Billingham, Cleveland, U.K.). Phenobarbitone sodium and remaining chemicals were from Merck Ltd (Lutterworth, Leics, U.K.) and were of the highest purity available.

Animals and treatments. Female Fischer F344/N rats, 6 weeks old, were bred on site. Female C57Bl/6 and DBA/2 mice (20–25 g) were from Harlan Olac (Oxford, U.K.). Where indicated, phenobarbitone (0.1% w/v) or acetone 1% v/v) were given in the drinking water for 7 days. β-Naphthoflavone or dexamethasone, suspended in tricaprylin (10 mg/mL), were administered (i.p.) at doses of 20 mg/kg and 50 mg/kg, respectively, for 3 days. Tamoxifen, dissolved in tricaprylin (40 mg/mL) was administered by oral gavage (40 mg/kg) for 4 days. In all instances, animals were killed 24 hr after the last dose. Liver homogenates (10% w/v) were prepared in ice-cold 0.25 M sucrose and washed microsomal preparations were prepared by differential centrifugation as described by Lake [17]. Microsomal protein was determined by the Lowry method using BSA standard [18]. Human livers were obtained from kidney transplant donors and breast tissue from mastectomies. Preparation of human liver and breast microsomes and the characterization of CYP-isoenzymic forms were carried out as described previously [19].

Tamoxifen metabolism and covalent binding. Reaction mixtures of 0.4 mL volume in 100 mM Tris-HCl buffer, pH 7.4, contained EDTA (1 mM), microsomal protein (0.25 mg) and [^{14}C]tamoxifen (1 μCi, dissolved in 1 μL ethanol) at the concentrations indicated. Following 5 min equilibration to 37°C, reactions were started by the addition of NADPH (1 mM). Incubations were for 15 min in a shaking waterbath. Reactions were stopped by the addition of 800 μL ice-cold DMSO in methanol (1 : 4 v/v) containing oestradiol benzoate (100 pmol) as internal standard. Tubes were vortex mixed, cooled to −20°C for 2 hr, then centrifuged (10,000 × *g* for 5 min). The supernatant was analysed by HPLC by a modification of the method of Lim *et al.* [20].

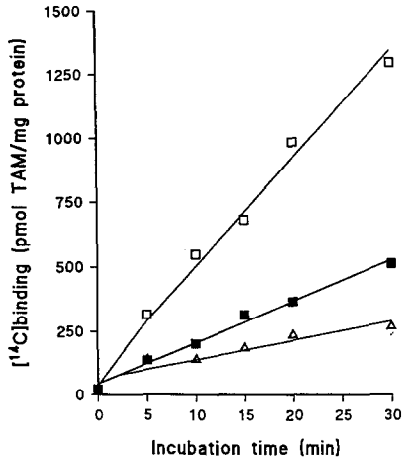

Fig. 1. Dependence on time of incubation on the covalent binding of [^{14}C]tamoxifen to liver microsomal preparations. Liver microsomes from female Fischer rats, DBA/2 mice or humans were incubated for the times indicated at 37°C in the presence of NADPH and [^{14}C]tamoxifen. Proteins were extracted with organic solvents as described in the Materials and Methods section and the ^{14}C radioactivity in the solubilized pellet determined. (□) Mice; (■) rats; (△) humans. Results represent the mean for two experiments.

A 15 × 0.4 cm LiChroCART, 5 μM end-capped column (E. Merck, Lutterworth, Leics, U.K.) was used with a linear gradient over 15 min of 5% aqueous ammonium acetate/methanol 3:7 (v/v) to 100% methanol at a flow rate of 1.3 mL/min. Detection was by UV absorption at 243 nm using a 966 photodiode array detector and Millenium integration system (Waters Ltd, Watford, U.K.). The identity of the metabolites was made on the basis of their retention times in comparison with authentic standards.

Determination of apparent K_m *and* V_{max} *values.* Determination of apparent K_m and V_{max} values was computed from weighted non-linear regression analysis [21] with a range of tamoxifen concentrations of 4.5–500 μM.

Covalent binding to microsomal proteins. For the determination of covalent binding, the protein precipitated from incubation mixtures was washed three times with DMSO/methanol then suspended in DMSO/methanol (1 mL), transferred to a 2.4 cm Whatman GF/C glass microfibre filter (Whatman Ltd, Maidstone, Kent, U.K.) and exhaustively washed with a series of organic solvents as described previously [12]. [^{14}C]radioactivity in final solubilised protein was determined by liquid scintillation counting following the addition of Hydrofluor scintillant (National Diagnostics). Conversion of cpm to dpm was computed using an external standard. Protein concentrations were determined as described above.

RESULTS

Characterization of the activation and covalent binding of [^{14}C]tamoxifen: comparison of rat, mouse and human liver microsomes

Table 1 compares the covalent binding of [^{14}C]-

Table 1. Characterization of covalent binding of [^{14}C]tamoxifen (45 μM) to microsomal proteins: comparison of rat, mice and humans

Species	Inhibitor	Covalent binding (pmol/15 min/mg microsomal protein)	
Human	–	26 ± 0.25	(10)
Rat	–	83 ± 2.5	(4)
Rat	No NADPH	30 ± 1.2	(4)
Rat	[^{14}C]Tamoxifen added after incubation	2.2 ± 0.3	(3)
Mouse	–	646 ± 15.0	(4)
Mouse	+ Ascorbate (1 mM)	450 ± 10***	(3)
Rat	+ Ascorbate (1 mM)	58 ± 2.7***	(4)
Rat	+ 2′AMP (5 mM)	32 ± 1.1***	(3)
Rat	+ Metyrapone (0.5 mM)	35 ± 1.8***	(4)

Liver microsomes from humans or female rats or mice were incubated with [^{14}C]tamoxifen for 15 min at 37°C. Covalent binding to microsomal protein was determined following extraction with organic solvents as described in the Materials and Methods section. The number of animals used is given in parenthesis. Probability of significance of difference between treated and control incubation mixtures not containing the inhibitor: ***, $P < 0.001$.

tamoxifen (45 μM) to rat, mouse and human liver microsomes. In all three species there was a linear increase in covalent binding to microsomal protein with time up to 30 min incubation (Fig. 1). Binding of [^{14}C]tamoxifen in female Fischer rats was 3-fold and DBA/2 mice 24-fold higher than in human liver microsomal preparations. The addition of ascorbate to the reaction mixtures results in a significant reduction in covalent binding of tamoxifen to mouse hepatic microsomes; however, a similar inhibitory effect was also seen in rats. Table 1 also shows that the binding of [^{14}C]tamoxifen to rat liver microsomal proteins was inhibited by the absence of NADPH or by the mixed function oxidase inhibitor metyrapone. Similarly, 2′-AMP brought about a significant reduction in the covalent binding of [^{14}C]tamoxifen, suggesting a requirement for cytochrome P450 reductase activity [22].

Comparison of the effects of concentration of [^{14}C]-tamoxifen on covalent binding to rat, mouse and human liver microsomes

Figure 2 shows that there are marked species differences in the covalent binding. Mouse liver microsomes had a very much lower apparent K_m value than rat microsomes. For these determinations, a time period of 15 min was chosen. This was on the linear part of the time response (Fig. 1) and with the [^{14}C] specific activity of tamoxifen available it permitted quantifiable determinations to be made down to low concentrations. For example, with 8 μM tamoxifen, following 15 min incubation with rat liver microsomes, in the presence of NADPH, bound radiolabel was in the order of 1000 dpm/mg microsomal protein. To minimize on the use of radiolabel, the linearity of the time course was not determined for every substrate concentration. Non-linearity might result in some overestimate of the apparent K_m and V_{max} values. In the case of human liver microsomes, rates of covalent binding were nearly linear up to 100 μM, beyond the aqueous solubility limit for tamoxifen, preventing accurate estimates of apparent K_m and V_{max} being obtained. Results in Fig. 2 suggest them to be of a similar order of magnitude to those of the rat (Table 2).

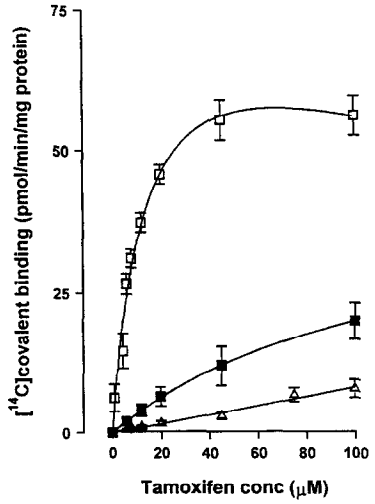

Fig. 2. Effects of concentration on the covalent binding of [^{14}C]tamoxifen to liver microsomal preparations. Liver microsomes from female Fischer rats, DBA/2 mice or humans were incubated for 15 min at 37°C in the presence of NADPH and [^{14}C]tamoxifen at the concentrations indicated. Proteins were extracted with organic solvents as described in the Materials and Methods section and the ^{14}C radioactivity in the solubilized pellet determined. (□) Mice; (■) rats; (△) humans. Results represent the mean ± SE for four experiments.

Effect of pretreating rats with monooxygenase inducers on the covalent binding of [^{14}C]tamoxifen to liver microsomes

In order to characterize the forms of cytochrome P450 involved in the metabolic activation of

Table 2. Comparison of kinetic parameters for covalent binding of [^{14}C]tamoxifen to rat, mouse or human liver microsomal preparations

Species	Apparent K_m (μM)	Apparent V_{max} (pmol/min/mg protein)
Rat	119 ± 41	36 ± 2
Mouse	9.6 ± 1.9	50 ± 2
Human	See text	

Liver microsomes from female Fischer rats, DBA/2 mice or humans were incubated for 15 min at 37°C in the presence of NADPH and [^{14}C]tamoxifen over a nominal concentration range of 4.5–500 μM. Proteins were extracted with organic solvents as described in the Materials and Methods section and [^{14}C]radioactivity in the solubilized pellet determined. Results represent the mean ± SE of a minimum of three experiments.

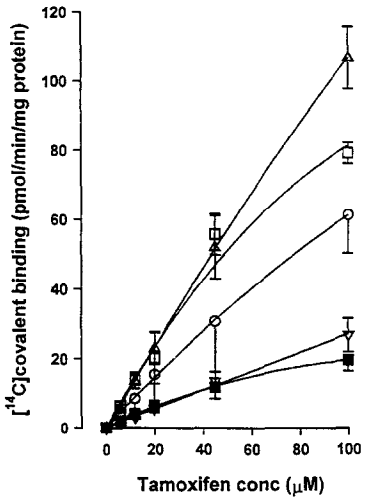

Fig. 3. Effect of pretreatment of rats with monooxygenase inducers on the covalent binding of [^{14}C]tamoxifen to liver microsomal proteins. Liver microsomal preparations from female Fischer rats pretreated with the monooxygenase inducers indicated were incubated with [^{14}C]tamoxifen at the concentrations indicated and processed as described in the legend to Fig. 1. Results represent the mean ± SE for three experiments. (■) Controls; (○) acetone; (△) phenobarbitone; (▽) β-naphthoflavone; (□) dexamethasone.

tamoxifen, the effect of pretreating rats with different inducers of cytochrome P450 enzymes was investigated. Figure 3 shows that pretreatment of rats with dexamethasone, phenobarbitone and to a lesser extent, acetone resulted in an induction of the covalent binding of [^{14}C]tamoxifen to microsomal protein, whereas β-naphthoflavone was without effect. Figure 4 (A) shows that N-demethylation of tamoxifen was markedly induced by dexamethasone pretreatment. N-oxidation was decreased whereas 4-hydroxylation was not significantly affected by the pretreatment procedures (Fig. 4 (B) and (C)).

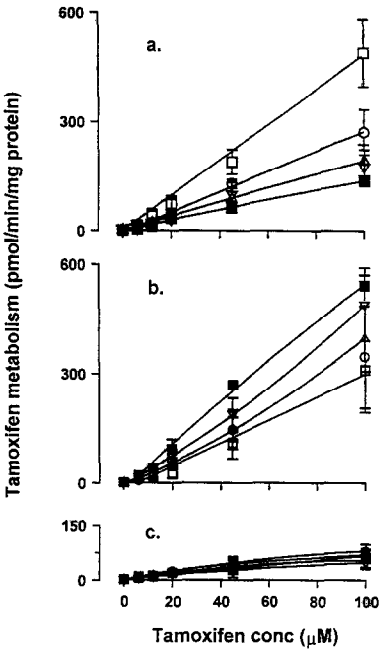

Fig. 4. Effect of pretreatment of rats with monooxygenase inducers on the N-demethylation, 4-hydroxylation and N-oxidation of tamoxifen. Rats were pretreated with the monooxygenase inducers as described in the Materials and Methods section. Liver microsomes were incubated with tamoxifen at the concentrations indicated and the concentration of metabolites estimated by HPLC. Results represent the mean ± SE for three experiments. (■) Controls; (○) acetone; (△) phenobarbitone; (▽) β-naphthoflavone; (□) dexamethasone. (a) *N*-demethyltamoxifen; (b) tamoxifen *N*-oxide; (c) 4-hydroxytamoxifen.

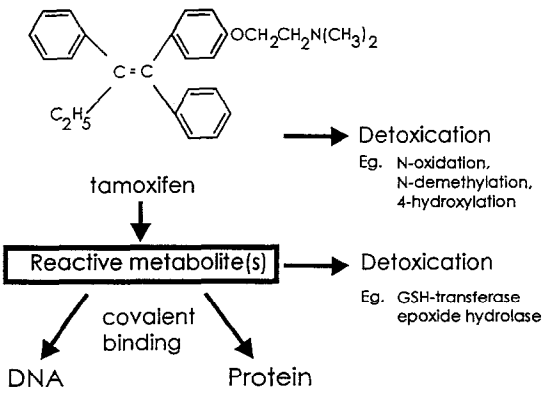

Fig. 5. Schematic diagram for the metabolic activation and detoxication of tamoxifen and its reactive metabolite(s).

Pretreatment of rats with tamoxifen itself resulted in a 4-fold increase in covalent binding of tamoxifen when this was added to a microsomal system *in vitro* (336 ± 180 pmol/15 min/mg protein). In contrast, in DBA/2 mice there was a much smaller 1.4-fold

increase (900 ± 105 pmol/min/mg protein, *cf.* Table 1).

Characterization of the human CYP-isoenzymes involved in covalent binding to liver microsomes

Table 3 shows there to be a statistically significant correlation between the concentration of CYP2B6 and 3A4 determined immunologically and the covalent binding of [^{14}C]tamoxifen to microsomal protein. In contrast, aromatic 4-hydroxylation showed the highest correlation with CYP2C9, although CYP2C8 and CYP2D6 may also be involved. Concentrations of tamoxifen in the livers of women taking tamoxifen therapeutically are in the range of 0.1–10 μM (Martin and White, in preparation). The study was therefore repeated using 8 μM [^{14}C]tamoxifen and a similar correlation between tamoxifen covalent binding and the content of CYP3A4 and 2B6 was obtained (data not shown). Microsomes from human breast tumour tissue were also able to activate tamoxifen (45 μM), although the level of covalent binding (3.45 ± 0.75 pmol/15 min/mg protein, mean ± SE, N = 6) was 7-fold lower than in human liver microsomes (*cf.* Table 1).

DISCUSSION

Characterization of cytochrome P450 isoenzymes involved in covalent binding of [^{14}C]tamoxifen to microsomal protein

A comparison has been made of liver microsomal activation of [^{14}C]tamoxifen to metabolite(s) which covalently bind to microsomal protein. Binding to protein was used as surrogate for DNA since preliminary studies showed DNA binding to be 30–50-fold lower than to protein (White, unpublished data) and this would not have permitted the kinetic studies to be carried out under the conditions described for the present study. The inhibition by metyrapone and 2′-AMP together with the inducibility by classical monooxygenase inducers, phenobarbital or dexamethasone, are consistent with the involvement of cytochrome P450 isoenzymes in the metabolic activation of tamoxifen. The omission of NADPH from the reaction mixtures containing washed rat liver microsomes, resulted only in a 60% reduction in covalent binding (Table 1), suggesting the possibility that enzyme systems other than the monooxygenases could be involved. However, a liver microsomal cytochrome P450 system has previously been described both for the activation of tamoxifen to give ^{32}P-postlabelled DNA adducts [23] and covalently bound protein adducts [12]. The present results show a much higher level of covalent binding to mouse than to rat liver microsomes. This is consistent with the higher overall rate of microsomal metabolism in mice compared to rats [20]. In the present study it is not possible to state if the dramatically different effectiveness of [^{14}C] tamoxifen binding to mouse microsomes can be explained by a higher content of P450 in the microsomes. One might expect that representation of the data on a per nmol of cytochrome P450 to be important. Comparisons, however, are difficult as authentic purified P450 standards from rat mice and humans are not available and P450 concentrations determined by Western blotting need not necessarily reflect catalytic activity. In addition all of the isoenzymic forms which contribute to binding have not yet been defined. In an investigation on species differences in the metabolism of digitoxin, which is CYP3A dependent, the order of activity was rat > mouse > human [24]. Comparison of basal rates of metabolism of different alkoxyresorufins show a general order of activity mouse > rat > human [25].

DNA damage *in vivo*, as determined by ^{32}P-postlabelling, following tamoxifen administration, is 3- to 4-fold lower in mice than in rats [9]. An epoxide metabolite has been proposed as the intermediate involved in damaging DNA [26]. A model tamoxifen epoxide prepared synthetically can react with rat liver DNA to give ^{32}P-postlabelled DNA adducts [27]. Mouse liver microsomes from more ring hydroxylated metabolites when incubated with tamoxifen than rats [20] and it has recently been suggested that 4-hydroxytamoxifen may be a proximate reactive intermediate of tamoxifen [28]. The possibility that phenoxy or (quinone) metabolites might be involved in covalent binding to protein was considered. Phenoxy radicals are intermediates in the oxidation of phenolic compounds to quinoid derivatives known to act as carcinogenic agents by reacting with macromolecular targets. Ascorbate reverses one electron oxidative activation of such compounds [29, 30]. Addition of ascorbate to the reaction mixture resulted in a similar reduction in covalent binding in both rat and mouse liver microsomal systems, suggesting the same activation mechanism in both species. The lower potential of rat, relative to mouse liver microsomes, to activate tamoxifen *in vitro* at pharmacologically relevant concentrations does not reflect the greater hepatic DNA damage [9] or the long-term hepatocarcinogenicity [3, 6] in rats. It cannot be distinguished if different reactive metabolites are involved in protein and DNA binding or if phase II pathways play a major role in detoxication of the reactive species (Fig. 4). This apparent conflict in the data needs to be clarified.

Covalent binding of tamoxifen in microsomes from breast tissue

The present results show that metabolic activation of tamoxifen can occur in breast tissue, although recent attempts to characterize the cytochrome P450 isoenzymes in breast tumours have not been successful [31]. Other organs were not investigated. The present results suggest that this needs to be carried out, particularly for endometrium since epidemiological evidence suggests that, in women, endometrial cancer may be a more frequent side-effect of tamoxifen than that in liver [7, 8].

Isoenzymic cytochrome P450 forms involved in tamoxifen activation

Covalent binding of tamoxifen showed a positive correlation with the content of CYP3A4 and 2B6 in human microsomal systems (Table 3). The concentration of the enyzmic protein estimated by Western blotting may not always be an accurate reflection of catalytic activity. However, the

Table 3. Human liver microsomes: ranking of cytochrome P450 content and covalent binding

CYP ranking	N-Demethylation	4-Hydroxylation	Covalent binding
1A1	−0.147	−0.688	−0.161
2A6	0.387	−0.426	0.434
2B6	0.503	−0.407	0.580*
2C9	0.133	0.651*	0.210
2C8	0.385	0.538	0.406
2D6	−0.385	0.592	−0.392
2E1	−0.385	0.314	−0.063
3A4	0.510	−0.041	0.713**
4A1	−0.392	0.009	−0.210

Ten human liver microsomal samples were incubated with [^{14}C]tamoxifen (45 μM) and NADPH for 15 min at 37°C. Mean rate of covalent binding is shown in Table 1. Rates of N-demethylation and 4-hydroxylation were 190 ± 90 and 215 ± 34 pmol/min/mg protein respectively. Probability of significance by Spearman correlation ranking: * $P < 0.05$, ** $P < 0.01$.

involvement of the analogous rat enzymes CYP3A1 and 2B1 is inferred from the stimulation of covalent binding we have now described following treatment of rats with dexamethasone or phenobarbitone, respectively (Fig. 3). The dexamethasone inducible CYP3A4 also appears to be the main isoenzymic form involved in the N-demethylation of tamoxifen, confirming previous observations [13, 32]. Comparative studies with toremifene using 32 human liver microsomal preparations have shown the CYP3A4 isoenzyme to be involved in N-demethylation of this tamoxifen analogue [33]. These authors found CYP1A not to be involved in the 4-hydroxylation of toremifene. In the present study, pretreatment of rats with β-naphthoflavone did not result in an induction of tamoxifen hydroxylation. However, in chick embryos, treatment with either this compound or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin resulted in an increased rate of formation of 4-hydroxytamoxifen [34]. It is not clear if the inducibility and specificity of avian P450s differs from that of mammals. In an earlier study, tamoxifen gave a positive micronucleus test using a human lymphoblastoid cell line expressing CYP2E1 [11]. In human microsomal systems, no evidence was obtained to indicate involvement of CYP2E1 in the activation and covalent binding of tamoxifen. The possibility that the addition of tamoxifen in ethanol vehicle might have caused some competitive inhibition of tamoxifen activation with the human microsomal systems cannot be excluded. This study investigated whether pretreatment of rats or mice with tamoxifen could result in the suicidal destruction of the cytochrome P450 activating enzyme, causing a loss of catalytic activity. If destruction of cytochrome P450 was greater in mice, this could account for their greater resistance to tamoxifen genotoxicity [9]. When microsomal preparations from tamoxifen pretreated animals were incubated with tamoxifen, there was an induction rather than an inhibition of covalent binding. This is, however, consistent with the induction of CYP3A1 and CYP2B1 seen in rats and in mice following pretreatment with this drug [35]. No attempt was made to determine the effects of these pretreatments on the binding of tamoxifen to membrane high-anti-oestrogen receptors that have now been purified to homogeneity [36, 37]. Non-covalent interactions are distinct from the covalent binding determined in this study. Binding to anti-oestrogen receptors might be associated with longer term neoplastic changes brought about by tamoxifen treatment.

Effects of tamoxifen concentration on covalent binding

The present results show there to be a large difference between mice and rats or humans in the effects of different tamoxifen concentrations on covalent binding. The female mouse enzyme is saturated at relatively low tamoxifen concentrations whereas in rat and human liver microsomal preparations, apparent K_m values are over an order of magnitude higher. Given sufficiently high tamoxifen concentrations, the extent of covalent binding to protein in rats, mice or humans might be expected to be of a similar order of magnitude. These results suggest that in humans and rats, following dosing *in vivo*, binding to protein (and DNA damage) will be dependent on the tamoxifen concentration in the liver. It has been demonstrated that in rats there is a linear relationship between hepatic DNA damage and tamoxifen dose over the range 5–45 mg/kg [9]. Similar studies have not been carried out in mice. However, following a single dose of 200 mg/kg, serum and liver concentrations of tamoxifen in mice were still considerably lower over a 72 hr period than in rats, suggesting that even at this high dose level, mice metabolize the drug more rapidly [38].

CONCLUSIONS

Species differences in the NADPH-dependent binding of [^{14}C]tamoxifen to liver microsomes suggest that the rat is a better model than the mouse for human liver microsomal metabolism of tamoxifen both with respect to kinetic parameters and the pattern of metabolites formed [26]. The evidence

suggests that the major form of cytochrome P450 involved in tamoxifen activation in humans is CYP3A4 although other isoenzymes, for example CYP2B6, may also be involved. The genetic diversity of drug metabolizing enzymes in the human population and the inducibility of the P450 isoenzymes will contribute to the response of the individual to the harmful side-effects of this drug.

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