EFFECT OF INHIBITORS ON THE BIOTRANSFORMATION OF TAMOXIFEN BY FEMALE RAT AND MOUSE LIVER SLICES AND HOMOGENATES

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SUMMARY

The metabolism of tamoxifen was studied in female Sprague-Dawley rat and mouse liver slices and homogenates, and the three principal tamoxifen metabolites, 4-hydroxytamoxifen, N-desmethyl-tamoxifen and tamoxifen N-oxide, were identified by HPLC using authentic standards. It was not possible to identify any of the minor metabolites such as the epoxides using this technique. The N-oxide metabolite only appeared when NADPH was added to the system; this is because the production of tamoxifen N-oxide is primarily mediated by microsomal flavin monooxygenase (FMO) which is NADPH dependent. However, this metabolite did appear in incubations with mouse liver slices only, because they are rich in flavin monooxygenases (FMOs). It did not appear in female rat or mouse liver homogenates, because the NADPH present is destroyed during homogenisation, therefore it was necessary to add NADPH to the system to produce the N-oxide metabolite.

The purpose of this study was to investigate the effect of inhibitors on the biotransformation of tamoxifen by female rat and mouse liver slices and homogenates. Female rat liver slices and homogenates were incubated with the following inhibitors (1 mM): cimetidine, ascorbate, sodium azide and reduced glutathione. Cimetidine, a general P-450 inhibitor, inhibited the production of the N-desmethyl metabolite by about 80%; this is in agreement with the action of the other inhibitors.

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Reduced glutathione, ascorbate and sodium azide are mainly peroxidase inhibitors, so therefore from these novel and interesting results it was possible to suggest that peroxidases play a role in the metabolism of tamoxifen. This observation was also strengthened when the production of the N-desmethyl metabolite increased when horseradish peroxidase was added to the incubate. The production of 4-hydroxytamoxifen was reduced and the N-oxide metabolite was completely inhibited in the presence of peroxidase inhibitors. When rat liver homogenates was incubated with superoxide dismutase (SOD) and catalase, it was observed that the N-desmethyl metabolite disappeared completely at 60 min and the N-oxide and 4-hydroxy metabolites were completely inhibited. However, this phenomenon was only observed when SOD and catalase were preincubated for 30 min with the rat liver homogenate at 37°C; without preincubation the production of these metabolites was unaffected.

Finally, the effect of long incubation periods (300 min) on the production of metabolites was examined. It was found that there was a reduction in the concentration of metabolite produced after 60 min which was due to enzyme and co-factor degradation.

KEY WORDS

tamoxifen, metabolism, rat, mouse, liver slices, homogenate, inhibitors, peroxidases

INTRODUCTION

The synthetic antioestrogen tamoxifen [Z-(l-(4-(2-dimethylaminoethoxy)phenyl-1,2diphenyl-l-butene)] is currently in clinical use for the treatment of oestrogen-dependent breast cancer /1,2/. In recent years, much evidence has emerged that metabolism may play an important role in modulating the activity of this compound /3/. Originally a phenolic derivative, 4-hydroxytamoxifen, was the only serum metabolite detected /4/, but later the major serum metabolite was shown to be the N-desmethyl derivative /5/. Hepatic microsomal metabolism studies showed a similar metabolic profile /6/ with the identification of tamoxifen N-oxide /7/ as a further metabolite. Subsequent hepatic microsomal metabolism studies revealed the

presence of three principle polar metabolites common to humans, rats and mice /8,9/: N-desmethyltamoxifen, 4-hydroxytamoxifen and tamoxifen N-oxide. Various antibodies to cytochrome P-450s, enzyme inducers and inhibitors have been used to determine the pathway involved in tamoxifen metabolism. Results indicate that the formation of tamoxifen N-oxide is primarily mediated by microsomal flavin monooxygenase (FMO) /10,11/, and the metabolite is then rapidly excreted or reduced back to the amine at an extremely slow rate. This observation has led to speculation that tamoxifen N-oxide represents a storage form of tamoxifen *in vivo,* and the reason it has remained undetected in human biological fluids is as a result of its conversion back to tamoxifen by endogenous reductants /12/. Both 4-hydroxy-tamoxifen and N-desmethyltamoxifen undergo microsomal oxidative metabolism involving several isoenzymatic forms of cytochrome P-450 /10,13/. 4-Hydroxylation is thought to be catalysed by inducible CYPIA, CYP2C and CYP3A enzymes in the rat, but just CYP3A in humans, whilst N-demethylation involves constitutive enzymes. Studies have suggested that tamoxifen metabolites may play an important role in mediating its pharmacological effects, and of particular interest is 4-hydroxytamoxifen, which has an affinity for the oestrogen receptor one hundred times that of tamoxifen and a correspondingly greater antitumour potency *in vitro* /14/. This high potency, however, is not reflected *in vivo* where the two have similar efficacy, correlating with the lower plasma levels of 4-hydroxy-tamoxifen as compared to tamoxifen, and attributed to a rapid deactiv-ation mechanism by way of glucuronide formation at the hydroxyl group /13/. It has also been proposed that the effects of tamoxifen which arise as a result of oestrogenic activity are due to its metabolic transformation into the *cis* (E) isomer which exhibits pure oestrogenic activity.

Significant quantitative and qualitative differences have been observed in the minor metabolites detected in different animal models. The production of various epoxide metabolites by liver microsomal preparations has been investigated /15/, since epoxides are known to be reactive species capable of DNA adduct formation and are widely implicated in carcinogenesis. This study showed that in addition to the major tamoxifen metabolites expected, rat liver microsomes produced two epoxide metabolites, 3,4-epoxytamoxifen and 3′,4′-epoxytamox-ifen and their hydrolysed derivatives, 3,4-dihydrodihydroxytamoxifen

and 3′,4′-dihydrodihydroxytamoxifen, as detected by on-line EPLC electrospray ionisation MS /15/.

The aim of this study was to examine the effect of peroxidases on the biotransformation of tamoxifen in rodent liver slices and homogenates.

MATERIALS AND METHODS

Chemicals

Tamoxifen [Z-(l-(4-(2-dimethylaminoethoxy)phenyl-1,2diphenyl-l-butene)], NADPH, cimetidine, sodium azide, reduced glutathione, ascorbate, horseradish peroxidase, superoxide dismutase, catalase and all other chemicals were purchased from Sigma Chemical Co. (Poole, UK). All solvents used were HPLC grade and were obtained from BDH Chemical Co. (Poole, UK). N-Desmethyltamoxifen was supplied by Zeneca (Cheshire, UK); 4-hydroxytamoxifen /16/, tamoxifen N-oxide /11/, N-oxide epoxide /14/, E-tamoxifen, /17/ and tamoxifen epoxide /18/ were synthesised according to published procedures in this laboratory. Female Sprague-Dawley rats and mice were obtained from breeders within the UK.

Preparation of liver slices and homogenates

Preparation of female rat and mouse liver slices, and liver slice incubations were carried out in Krebs-Henseleit buffer (pH 7.4) made from a concentrate which was stable at 4°C for up to 3 months. The composition of the concentrate was as follows: NaCl (1.18 mM), $CaCl_2.6H_2O$ (2.2 mM), KCl (4.75 mM), $MgSO_4$ (1.2 mM) and KH_2PO_4 (1.2 mM). Buffer was made up daily by adding 20.0 ml of the concentrate and 1.05 g sodium hydrogen carbonate to 450 ml of distilled water. The pH was adjusted to 7.4 with 0.1 M HCl and the buffer volume was made up to 500 ml with distilled water. Female rats and mice were killed between 9.00 am and 10.00 am by cervical dislocation, the livers were immediately excised and placed in ice-cold Krebs-Henseleit buffer (pH 7.4), which was continuously oxygenated with 95% oxygen/5% carbon dioxide. The liver slices were prepared using a manual slicer which was manufactured in the department. This slicer was comprised of a tissue bore, holder and microtome blades.

Cores, 1 cm in diameter and approximately 1 cm in depth, were bored from the liver lobe using the tissue borer. The core, saturated with Krebs-Henseleit buffer (pH 7.4), was then placed inside the die, and a Perspex cover placed over the top. The screw was turned so that the core was pushed up against the Perspex cover, and slices were produced by sliding the microtome blade between the Perspex cover and the base of the die. The thickness of the slices was determined by the rotation of the screw. One third of a rotation gave slices approximately 0.3 μm thick. The first and the last slices from each core were discarded because they contained the liver capsule. Slices were placed in Krebs-Henseleit buffer (pH 7.4) saturated with oxygen/carbon dioxide until slice preparation was complete. Slices were then immediately used for incubations.

Rat and mouse liver homogenates were produced by homogenising approximately 10 g liver in 35 ml of 1.15% KCl solution containing 0.1 mM EDTA. Approximately 0.5 ml homogenate is equivalent to four liver slices.

Preparation of standard solutions of tamoxifen and metabolites

A standard solution of tamoxifen was made up (200 μM) in dimethylsulphoxide (DMSO) or methanol and a series of dilutions (0-200 μM) was prepared. The calibration curve produced was found to be linear within this range (r^2 = 0.999). Tamoxifen is insoluble in aqueous solvents such as Krebs buffer (pH 7.4), therefore an appropriate amount (8 mg) was weighed out and dissolved in a small volume (1 ml) of dimethylsulphoxide (DMSO) or methanol. 20 μl of this solution was then added to the incubation (3 ml) to give a final concentration of 143 μM. It was very important to keep the percentage of organic solvent as low as possible (<1%) in the incubation, as this may poison the enzyme systems present. Metabolism studies were carried out on a range of substrate concentrations (50-200 μM), and it was found that the concentration of metabolites produced remained constant above 143 μM, so therefore 143 μM was chosen for tamoxifen metabolism studies. Metabolite and substrate concentrations were expressed in micromoles (μM) instead of mg/l or μg/ml, because the parent compound is made up in organic solvent and buffer (pH 7.4) and μM is defined as weight of compound per volume of solution. By expressing concentration in μM, it is easier to compare results in

buffer only with those obtained in the presence of enzymes; it is the most appropriate unit to use for *in vitro* studies.

4-Hydroxytamoxifen, N-desmethyltamoxifen and the N-oxide metabolite produced linear calibration curves within the range 0-50 μM ($r^2 = 0.999$). Like tamoxifen, the above solutions were insoluble in aqueous solvent so therefore they were made up in methanol or DMSO.

The purity of each purchased compound was checked by HPLC using diode array detection and found to be 100%. Their structural identity was confirmed by spectroscopic analysis, i.e. NMR and mass spectrometry. Each synthesised compound was purified by column chromatography and their purity was then checked as described above and found to be 100%.

Tamoxifen metabolism studies

Tamoxifen (143 μM) was incubated in Krebs-Henseleit buffer (pH 7.4) to a total volume of 3 ml at 37°C for 60 and 300 min. Incubations were performed in 10 ml closed vials which had holes drilled in their caps for the purpose of oxygenation at regular intervals throughout the incubation period. Oxygen is needed for oxidative metabolism. These were then placed in a shaking water-bath and pre-warmed to 37°C before initiation of the reaction by the addition of four liver slices or 0.5 ml of hepatic homogenate. The vials were wrapped in tin foil and the water-bath was kept covered throughout the incubation period; this was done because tamoxifen is light sensitive. A separate vial was used for each time point in the case of the liver slices; this is because some metabolites may adhere to the slices. This problem was overcome by homogenising the slices at each time point, centrifuging and then sampling. Aliquots (100 μl) were taken at 1, 5, 10, 20, 60, 120, 180 and 300 min time intervals using a Gilson micropipette. It was not possible to sample at time zero, because the sample was homogenised for 1 min. Incubations containing liver homogenate were carried out in the presence of NADPH (0.5 mM; 0.5 ml). Incubations were also performed in the presence of the following compounds (ImM): cimetidine, sodium azide, reduced glutathione and ascorbate. Catalase, superoxide dismutase, horseradish peroxidase (1,000 units per ml, pH 7.4; 0.5 ml) were used as enzymes in rat liver homogenate incubations. Control incubations were carried out along with normal incubations, these contained no tamoxifen, no NADPH, no tissue, and

no inhibitors, respectively. These were necessary to validate the results obtained with normal incubations. Standards of tamoxifen and metabolites were also run with each set of incubations. Aliquots (100 µl) were removed at various time intervals and added to 200 µl of 5% DMSO/methanol to terminate the reaction. Samples were centrifuged in a Beckman bench-top microcentrifuge at 9,000 rpm for 5 min, and the supernatant was subsequently analysed by HPLC. This technique was used to monitor the appearance and disappearance of each metabolite.

The effect of long incubation periods (300 min) on the production of metabolites was also examined. It was found that there was a reduction in the concentration of metabolite produced after 60 min, which was due to enzyme and co-factor degradation. Therefore an incubation period of 60 min was chosen as the most appropriate duration.

HPLC analysis

HPLC analysis was carried out using a system supplied by Waters Associates, Northwich, Cheshire which consisted of a 717 plus Autosampler, UV 486 Tunable Absorbance Detector and a 510 pump, controlled by Millenium software. All incubations were analysed by HPLC using a Spherisorb 5ODS1, 5 µM (25 cm x 4.6 mm i.d.) column with a µBondapak C18 Guard-Pak insert. The mobile phase consisted of methanol/acetonitrile/water/trichloroacetic acid (pH 2.9) (50:31:19:0.1% v/v) at a flow rate of 1.0 ml/min. This mobile phase was developed in our laboratory /19/. The reactions were monitored at 280 nm and room temperature. It was clear from the chromatograms produced that the retention times of each compound remained fairly constant throughout the day. This also applied on an interday basis. Metabolite concentrations were calculated (µM) with respect to standards synthesised in the laboratory. The metabolites had the following retention times: tamoxifen (21 min), 4-hydroxytamoxifen (9.5 min), N-desmethyltamoxifen (15 min), tamoxifen N-oxide (16 min) and E-tamoxifen (19 min).

Peak purity analysis

Peak purity analysis was carried out using a system supplied by Severn Analytical, Macclesfield, Cheshire which consisted of a Severn

Analytical Autosampler, a diode array detector and a LC-9A Shimadzu liquid chromatograph pump all controlled by GynkoSoft software. The chromatographic conditions used were similar to those already described in the HPLC analysis section. All peaks were found to be pure when analysed using a diode array detector.

RESULTS

Metabolism of tamoxifen by female rat liver slices and homogenate in the presence and absence of NADPH and also in the presence of inhibitors

Metabolic profiles were drawn showing the formation of each metabolite over 60 and 300 min at 37°C and under various conditions. When tamoxifen (143 μM) was incubated with Sprague-Dawley female rat liver slices only (mean of 5 separate animals) for 300 min, the production of the N-desmethyl metabolite was very rapid initially. There was a very gradual increase in the production of this metabolite during the first 120 min, then a substantial decrease up to 300 min (Fig. 1a). The 4-hydroxy metabolite appeared after 1 min, and in-

(a)

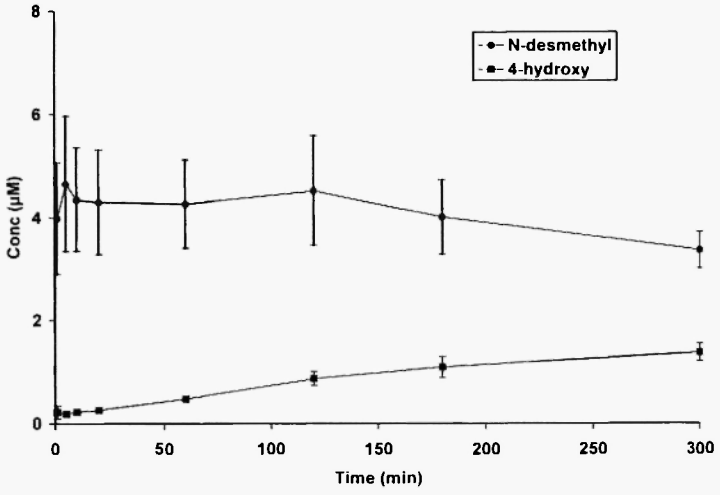
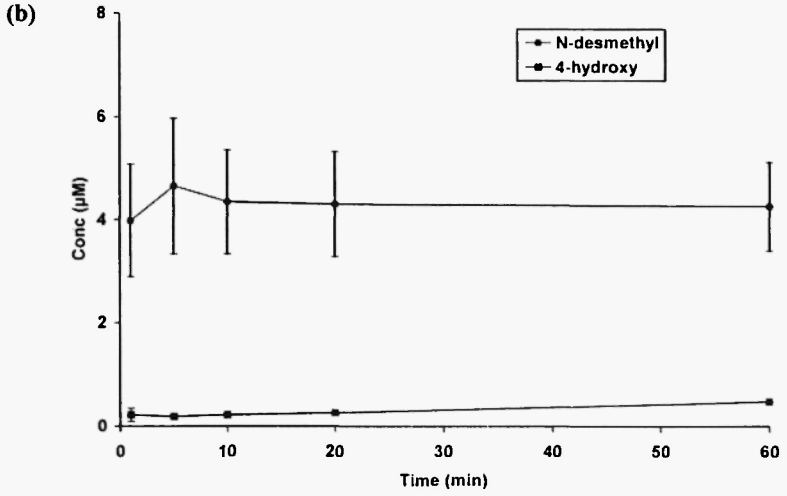


Fig. 1.



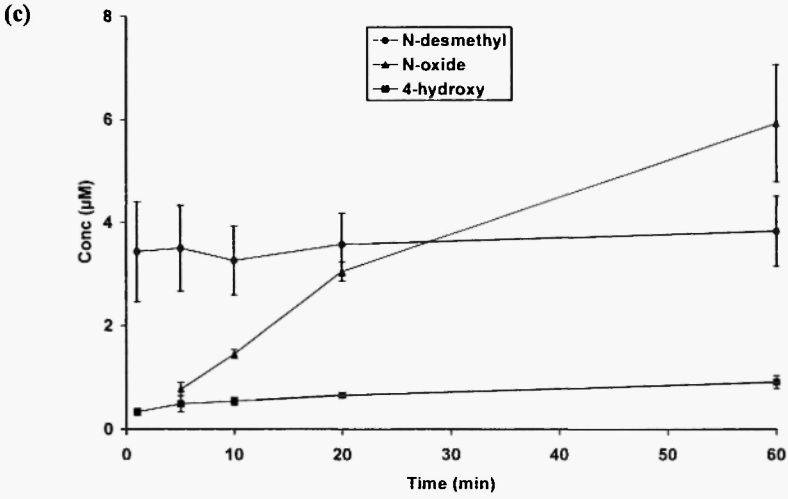


Fig. 1: Profiles showing the formation of metabolites after tamoxifen (143 μM) was incubated with female rat liver slices only at 37°C for **(a)** 5 h, **(b)** 1 h and **(c)** 1 h in the presence of NADPH. Mean ± SEM (n = 5), mean of 5 separate animals.

creased gradually over the 300 min period (Fig. 1a). The tamoxifen N-oxide metabolite only appeared after the NADPH cofactor was added to the incubate (Fig. 1c). One would expect the appearance of this metabolite without the addition of the NADPH, as this cofactor is retained in intact liver slices. The presence of the NADPH did not affect the production of either the N-desmethyl or the 4-hydroxy metabolites. When cimetidine, a general P450 inhibitor, was included in the incubation, the N-desmethyl metabolite was inhibited by 80% (Table 1). There was complete inhibition of the N-oxide metabolite in the presence of cimetidine, and the production of the 4-hydroxy metabolite was inhibited by 60% (Table 1). When tamoxifen was incubated with female rat liver homogenate, there was ~40% decrease in the production of the N-desmethyl compared to that in liver slices (Fig. 2a-b). Similarly, the metabolic profile showed a decrease in the production of this metabolite after ~60 min. As one would predict, the formation of the N-oxide metabolite required the addition of the NADPH cofactor to the incubation, as this cofactor is damaged during homogenisation (Fig. 2c). There was more N-oxide produced in the rat liver slices than in the homogenate, and the production of the 4-

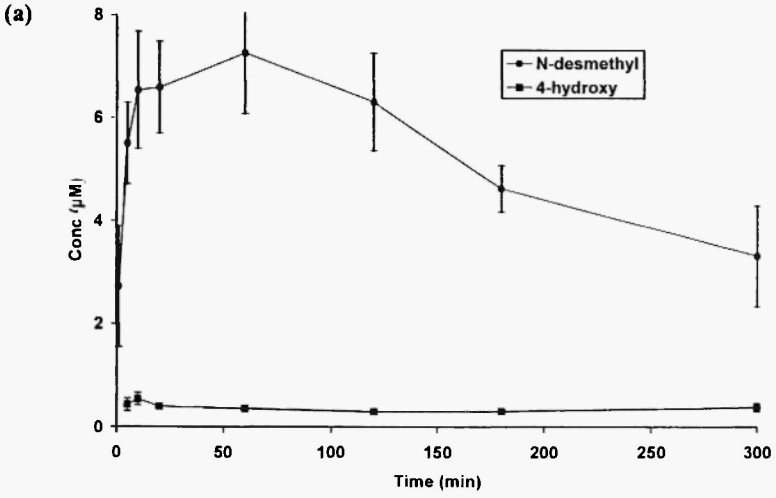
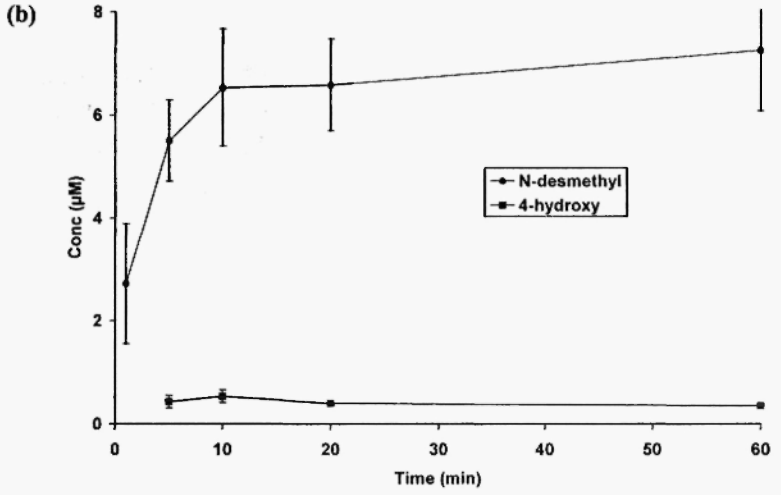


Fig. 2.



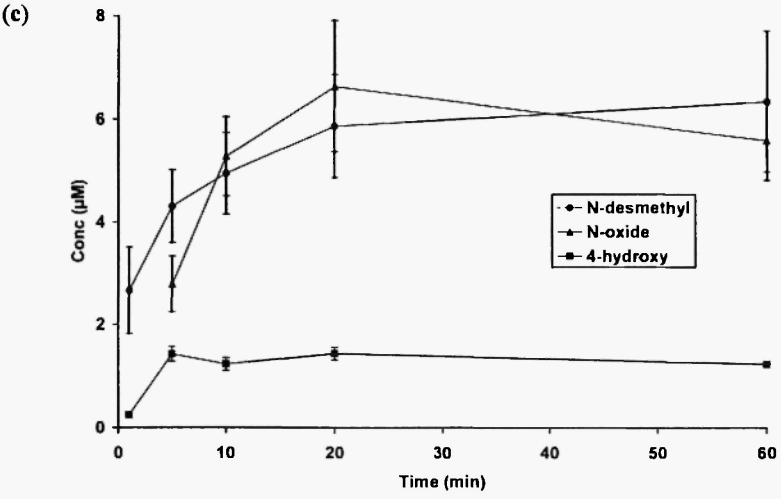


Fig. 2: Profiles showing the formation of metabolites after tamoxifen (143 μM) was incubated with female rat liver homogenate only at 37°C for (**a**) 5 h, (**b**) 1 h and (**c**) 1 h in the presence of NADPH. Mean + SEM (n = 5), mean of 5 separate animals.

hydroxy metabolite appeared to be enhanced by the presence of NADPH in the homogenate (Fig. 2c). The production of the N-desmethyl metabolite was substantially inhibited when any of the following inhibitors (1 mM), reduced glutathione, ascorbate or sodium azide, was added to the female rat liver homogenate incubates (Table 1). The production of the N-oxide was completely inhibited, and there was a sharp decrease in the production of the 4-hydroxy by the presence of the above inhibitors.

When female rat liver homogenate was incubated with catalase or superoxide dismutase (SOD) (1,000 units per ml), there was complete inhibition of the N-oxide metabolite formation after the enzyme was preincubated with the homogenate for 30 min. Without preincubation, the production of the N-oxide metabolite was unaffected. The N-desmethyl metabolite was completely inhibited by the presence of either enzyme after 60 min; this phenomenon only occurred when the enzyme was preincubated with the rat liver homogenate for 30 min. Horseradish peroxidase catalysed the N-desmethylation of tamoxifen.

TABLE 1

Percentage inhibition of each metabolite in rat liver homogenate

Inhibitor (1 mM)	**N-Desmethyl-tamoxifen**	**4-Hydroxy-tamoxifen**	**N-oxide-tamoxifen**
Cimetidine	80	60	100
Reduced glutathione	60	74	100
Sodium azide	55	80	100
Ascorbate	74	80	100

Values are presented as means (n = 5).

When rat liver homogenate was incubated with superoxide dismutase (SOD) and catalase (1,000 units per ml [pH 7.4]), it was observed that the N-desmethyl metabolite disappeared completely at 60 min and the N-oxide and 4-hydroxy metabolites were completely inhibited. However, this phenomenon was only observed when SOD and catalase were preincubated for 30 min with the rat liver homogenate at 37°C; without preincubation, the production of those metabolites was unaffected. Horseradish peroxidase (1,000 units per ml [pH 7.4]) catalysed the N-desmethylation of tamoxifen at 37°C.

Metabolism of tamoxifen by mouse liver slices only and homogenates in the presence and absence of NADPH

When tamoxifen (143 μM) was incubated with mouse liver slices only, the three main tamoxifen metabolites were produced. The N-oxide metabolite was produced, which is in contrast to that observed with rat liver slices only (Fig. 3a-b). There was a great enhancement in the production of the N-oxide metabolite following the addition of the NADPH cofactor to the mouse liver homogenate incubation (Fig. 4c). This regenerating system is destroyed during homogenisation, therefore the N-oxide metabolite is not produced in mouse liver homogenate-only incubations (Fig. 4a-b). The amount of N-desmethyl metabolite produced at t_0 in mouse liver slices was less than in rat liver slices, and also there was ~50% reduction in N-desmethyl formation after 60 min up to 300 min in mouse liver slice or homogenate incubations. This is in contrast to what was observed in rat liver incubations, in which this reduction was only observed after the addition of an inhibitor to the incubate.

There was ~5% conversion of Z-tamoxifen to E-tamoxifen in all incubations, the latter being a geometric isomer that is produced when the Z-isomer is exposed to light. The E-isomer was identified by HPLC and its concentration calculated with respect to an authentic standard. Confirmation of the presence of the E isomer of tamoxifen was achieved by using a mobile phase of acetonitrile and tetrahydrofuran (THF) in water and ammonia (300 + 75 + 125 + 2 v/v, pH 3.0), according to the method described in /20/.

DISCUSSION

The enzymes responsible for the metabolism of chemical carcinogens are primarily located bound to the endoplasmic reticulum in eukaryotic cells. The initial introduction of oxygen into the compounds is usually catalysed by the family of NADPH dependent cytochrome P-450 enzymes. Cytochrome P-450 enzymes are the final participants in an electron transport chain, the principle function of which is to hydroxylate a profusion of substrates. They are mixed-function oxidases and, as such, by obtaining two electrons from NADPH, can cleave molecular oxygen, incorporating one atom into water, and the other into the substrate /21/.

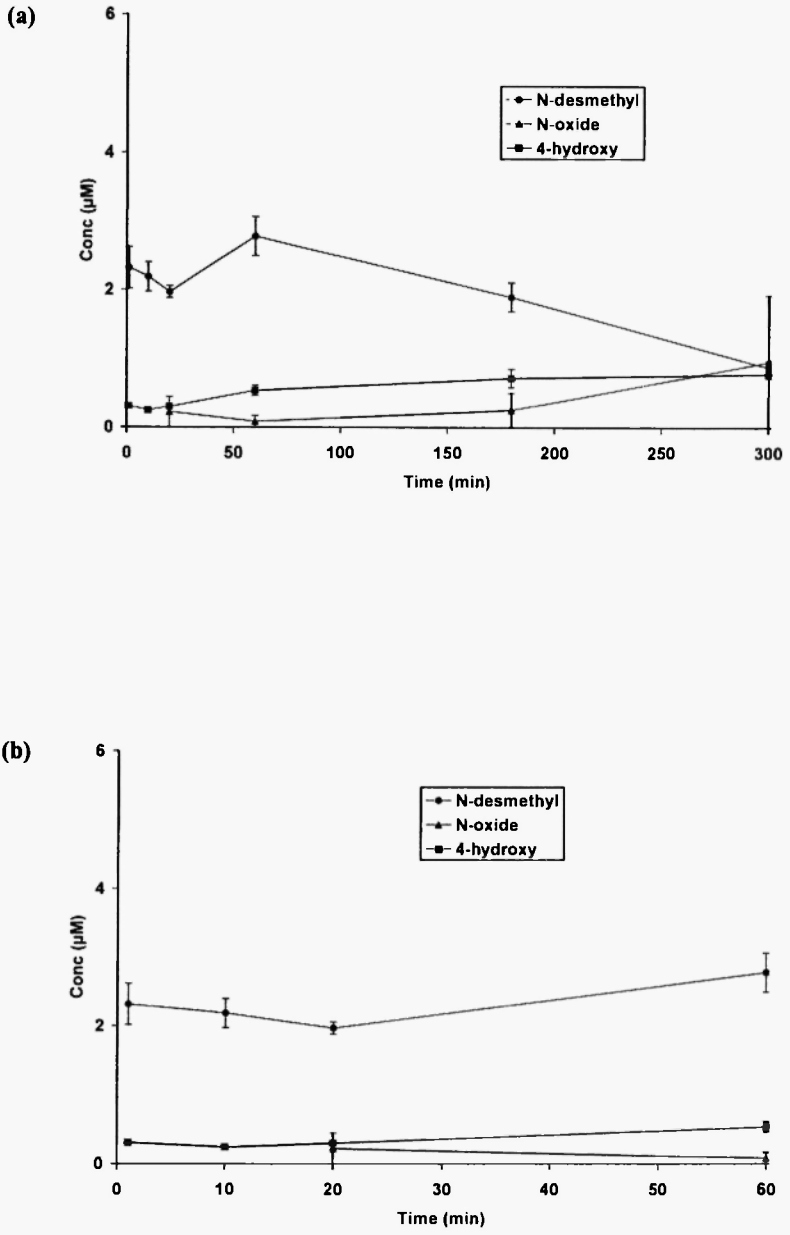


Fig. 3: Profiles showing the formation of metabolites after tamoxifen (143 μM) was incubated with female mouse liver slices only at 37°C for (**a**) 5 h and (**b**) 1 h. Mean ± SEM (n = 5), mean of 5 separate animals.

When the results described above are analysed, it is obvious that the production of the N-oxide metabolite is NADPH dependent. In rat liver slices only incubations, the N-oxide metabolite was not produced until NADPH was added to the system. This is in contrast to what happens with mouse liver slices only, in which the N-oxide is produced without the addition of NADPH. This occurs because mouse liver is rich in flavin monooxygenase (FMO), and the formation of tamoxifen N-oxide is primarily mediated by microsomal FMO /10/. This production was enhanced 5-fold by the addition of NADPH to the incubation. Some peculiarities of the FMO-catalysed N-oxidation of tamoxifen in rat liver have been noted /10/. Octylamine, a positive effector for FMO in mouse liver /22/, but exhibiting little effect or inhibition in some other species /23/, inhibited N-oxide accumulation. Surprisingly, pH 8.6, thought to be optimal for FMO /24/, was less effective than pH 7.4. N-Desmethyl and 4-hydroxytamoxifen are primarily formed by NADPH-dependent cytochrome P450 enzymes /8/. In the case of rat liver slices, the N-oxide metabolite may well be formed but reducing enzymes may catalyse the reverse reaction back to tamoxifen. It may be that only when supplemental NADPH is added does the forward reaction prevail /23/. In additional, it is possible that FMO, which is known to be thermally unstable, is not amenable to liver slice preparation /23/.

Both 4-hydroxytamoxifen and N-desmethyltamoxifen undergo microsomal oxidative metabolism involving several isoenzymatic forms of cytochrome P450 /8,13/

From the metabolic profiles obtained for the production of the N-desmethyl metabolite in mouse and rat liver slices and homogenates, the following assumptions can be made. The initial production of the N-desmethyl metabolite is possibly due to a peroxidase/H_2O_2 enzyme system. After about 120 min, the metabolic profile started to decrease up to 300 min; this is due to enzyme and/or cofactor degradation. The reasoning behind the above assumptions is that when a general P450 inhibitor, cimetidine (1mM), was incubated with female rat liver homogenates, the initial production of the N-desmethyl metabolite was inhibited by 80%. Similarly, when peroxidase inhibitors, sodium azide, reduced glutathione or ascorbate (1 mM), were added to the rat liver homogenate incubations, this initial production of N-desmethyl metabolite was substantially inhibited (Table 1). This was further verified when horseradish peroxidase catalysed the N-desmethylation

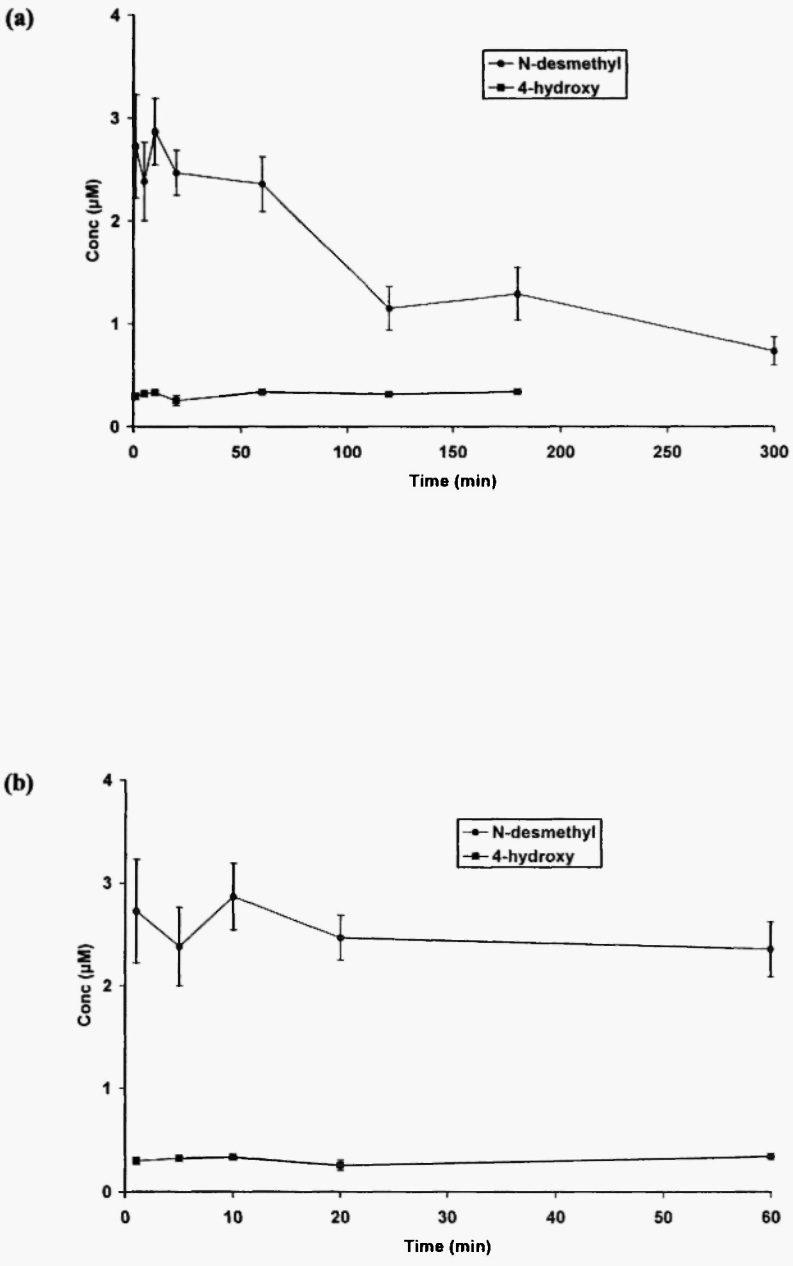


Fig. 4.

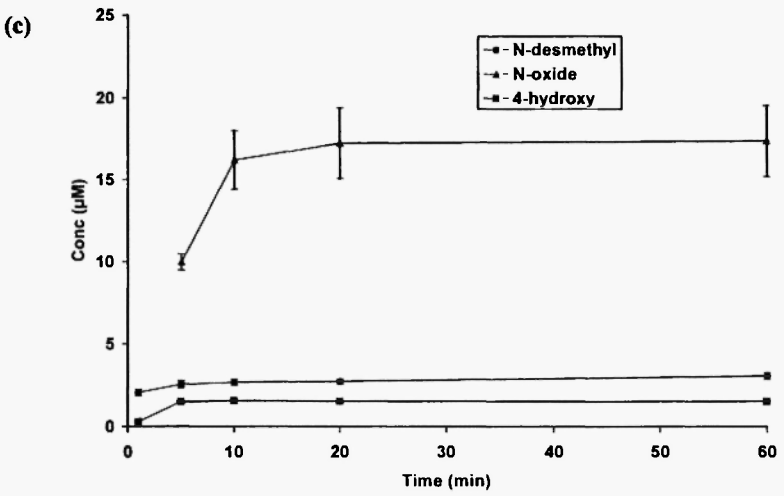


Fig. 4: Profiles showing the formation of metabolites after tamoxifen (143 µM) was incubated with female mouse liver homogenate only at 37°C for (**a**) 5 h, (**b**) 1 h and (**c**) 1 h in the presence of NADPH. Mean ± SEM (n = 5), mean of 5 separate animals.

of tamoxifen. In the presence of all the inhibitors, the production of the N-oxide metabolite was completely inhibited due to the absence of hydrogen donors (Table 1). Therefore, one can conclude that peroxidases play an important role in the metabolism of tamoxifen. Glutathione and ascorbate inhibit N-demethylation of crystal violet by the formation of thiol and ascorbate radicals, respectively /25,26/.

When rat liver homogenates were preincubated with superoxide dismutase (SOD) and catalase for 30 min, there was no N-oxide produced at all; in addition, the N-desmethyl metabolite disappeared completely at 60 min. This phenomenon was not observed without preincubation. This inhibition occurred because superoxide dismutase and catalase became inactive hydrogen donors during preincubation and had no effect on the NADPH reductase activity /27/.

Some reasonable speculatory comments on the mechanism of anti-peroxidase induced inhibition of tamoxifen metabolism can be put

forward. It is possible that the peroxidase inhibitors become bound to any intermediates formed, thereby preventing the formation of the respective metabolites. This would result in the formation of an inhibitor-intermediate complex whose structure could be determined using high performance liquid chromatography attached to a mass-spectrometer (LC-MS). This complex may be very stable and non-reactive, thereby preventing metabolite formation.

In conclusion, the three principal tamoxifen metabolites were identified: N-desmethyltamoxifen, tamoxifen N-oxide and 4-hydroxy-tamoxifen; the minor metabolites such as the epoxides could not be identified with only HPLC. However, this study produced some interesting and useful novel data which suggest a role for peroxidases in the biotransformation of tamoxifen by rodent liver slices and homogenates. This was confirmed by the action of the peroxidase inhibitors, reduced glutathione, ascorbate and sodium azide, shown in Table 1, on the production of the N-desmethyl metabolite. This was further verified when the N-desmethylation reaction was catalysed by horseradish peroxidase. The production of 4-hydroxytamoxifen was reduced and the N-oxide metabolite was completely inhibited in the presence of peroxidase inhibitors The effect of enzyme and cofactor degradation becomes apparent after approximately 60 min; this is shown by a decrease in the production of metabolites in the absence of inhibitors.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the Biotechnology and Biological Sciences Research Council (BBSRC) and the Yorkshire Cancer Research Campaign (YCRC) for this research.

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