Investigation of the formation and accumulation of liver DNA adducts in mice chronically exposed to tamoxifen

Elizabeth A. Martin1,2, Philip Cartwheel1, Ian N. H. White1, Robert T. Heydon1, Margaret Gaskell1, Robert J. Mauthe1, Kenneth W. Turteltaub2 and Lewis L. Smith1

1MRC Toxicology Unit, Hodgkin Building, University of Leicester, Leicester LE1 9HN, UK and 2Molecular Toxicology Group, Biology and Biotechnology Research Program and Center for AMS, Lawrence Livermore National Laboratory, University of California, Livermore, CA 94550, USA

To whom correspondence should be addressed

Tamoxifen was administered to three strains of female mice (B6C3F1, C57BL/6 and DBA/2) in short- and long-term studies to determine their ability to activate tamoxifen and cause hepatic DNA damage. 32P-Postlabelling of liver DNA from mice treated for 4 days showed a group of major adducts that increased in a dose-dependent manner and co-chromatographed with the major adducts detected in rat liver. On cessation of dosing, majority of the dosing, were cleared within 3 days. Binding of [14C]tamoxifen to DNA nucleotides was demonstrated by the use of accelerator mass spectrometry. In long-term studies of 12 months to 2 years duration, dependent on strain, tamoxifen was administered continuously in the diet to give a daily dose of ~40 mg/kg. DNA adducts were detected after 3 months, although the number of adducts decreased with time and by 2 years were not detectable in the tamoxifen treated mice. None of the treated groups showed a significantly increased incidence of liver tumours, with or without phenobarbital promotion and there was no sustained liver cell proliferation. Tamoxifen was detected in the mouse livers, but at levels 50 times lower than those reported in a comparable rat study. These results suggest that, in contrast to the rat, tamoxifen is non-carcinogenic in mice because it does not cause sufficient cumulative DNA damage, or act as a promoter by causing cell proliferation.

Introduction

The anti-oestrogen, tamoxifen (Z-1-[4-(dimethylaminoethoxy)-phenyl]-2-diphenyl-1-butene), is extensively used in the treat-ment of breast cancer (1). Clinical trials are underway to assess the potential of using tamoxifen as a chemopreventive agent in women with increased risk of developing the disease (2). Both short-term (3) and long-term tamoxifen treatment of rats leads to the development of hepatocellular carcinomas (4–7), which are accompanied by the accumulation of large numbers of DNA adducts in this organ, plus an increase in cell proliferation (7,8). Only one long-term study has previously been performed in the mouse (9) and in this study mice dosed with tamoxifen (5 or 50 mg/kg per day) for up to 15 months did not develop hepatocellular carcinomas. The study was terminated after 15 months because of the development of skeletal abnormalities (kyphosis) in many of the mice. DNA adducts have been detected in mouse liver following dosing with tamoxifen by gavage (10,11) and i.p. (12). C57BL/6 and DBA/2 mice treated by gavage with tamoxifen (45 mg/kg) for 4 days gave hepatic adduct levels of 17 and 28 adducts/10⁸ nucleotides respectively, which is approximately one-third of those detected in rats given similar doses of tamoxifen (10). In a separate study, ICR mice treated by gavage with tamoxifen (45 mg/kg) for 4 days gave hepatic adduct levels equivalent to three adducts/10⁸ nucleotides (11).

One characteristic of the DNA damage observed in three strains of rats following long-term exposure to tamoxifen is that the 32P-postlabelled DNA adducts accumulate over a 6-month treatment period to levels of ~3000 adducts/10⁸ nucleotides (3,7). In order to establish if mouse accumulate liver DNA adducts following long-term treatment, tamoxifen was administered to female DBA/2, C57BL/6 and B6C3F1 mice in the diet for up to 2 years. Three strains of mice were used because tamoxifen causes kyphosis in treated mice and this has limited the duration of previous bioassays. Using multiple strains increased the chance of finding a strain resistant to this effect, which would allow a full-term 2-year bioassay. This would determine whether mice are resistant to the carcinogenic effects of tamoxifen, or if in previous experiments mice have not been exposed for a sufficient time to give rise to liver tumours. The metabolism of tamoxifen in mice was investigated by measuring the presence of tamoxifen and its metabolites in serum and liver. 32P-Postlabelled adducts were measured in mouse liver to determine whether adducts accumulate during long-term tamoxifen treatment. The nature of the major DNA adducts was investigated by examining hepatic DNA nucleotides from [14C]tamoxifen treated mice using the sensitive technique of accelerator mass spectrometry (AMS*); and the half-life of adducts was determined by post-labelling. Hepatic cell proliferation was measured to assess whether tamoxifen was acting as a promoter of hepatic tumour formation. Understanding why mice are resistant to the carcinogenic effects of tamoxifen will contribute to an understanding of the mechanism of how tamoxifen causes tumours, this in turn will contribute information to the hazard assessment in humans.

Materials and methods

Chemicals

Tamoxifen (>98% pure as assessed by capillary GLC), was a gift from Dr J. Topham (Zeneca plc, Macclesfield, UK). Ring [14C]tamoxifen (sp. act. 2.03 GBq/mmol), of >98% radiochemical purity was from Cambridge Research Biochemicals (Billingham, Cleveland, USA). T4 Polydeoxynucleotide kinase (3’-phosphatase-free) and calf spleen phosphodiesterase were from Boehringer Mannheim (Leves, East Sussex, UK). Tricaprylin, micrococal nuclease, potato apyrase (grade VI), dithiothreitol, spermidine, ribonuclease A and T1 were from Sigma Chemical Co. (Poole, Dorset, UK). Water saturated phenol was from Rathburn Chemicals (Peeblesshire, Scotland, UK). [γ-32P]ATP (>185 TBq/mmol, 370 MBq/ml) was from Amersham International (Amersham, Buckinghamshire, UK). PEI-cellulose thin layer plates were from Macherey Nagel (Supplied by Camlab, Cambridge, UK). The remaining chemicals of

*Abbreviations: AMS, accelerator mass spectrometry; PB, phenobarbital; HPLC, high performance liquid chromatography; TLC, thin layer chromatography; PCNA, proliferating cell nuclear antigen; Tam, tamoxifen.
the highest available quality, except where indicated, were from Merck Ltd (Lutterworth, Leicestershire, UK) or Fisher Scientific Ltd (Loughborough, Leicester, UK).

**Animals and treatments**

**Long-term study.** Female DBA/2, C57BL/6 and B6C3F1 mice were purchased (at 4 weeks of age) from Harlan Olac (UK) Limited. Mice were housed three to a cage in isolators for the 4 weeks between purchase and experimental exposure. They were fed RM 1 diet supplied by SDS (Special Diet Services). Housing during the study was the same. At 8 weeks of age mice were fed either powered control diet (RM I) (48 mice per strain) or one containing tamoxifen (0.42 g tamoxifen/kg diet) (48 mice per strain). The tamoxifen diet was made up monthly, protected from light and assayed for tamoxifen content using HPLC as described previously (13). The daily dietary intake of B6C3F1 and C57BL/6 mice was ~120 mg/kg of tamoxifen and the daily dietary intake of DBA/2 mice was ~70% of that of the other two strains. After 8 weeks of feeding, the dietary amount of tamoxifen was reduced to one-third (0.14 g tamoxifen/kg diet) for the remainder of the study, to give a daily intake equivalent to ~40 mg/kg. This was to reduce the risk of kyphosis occurring in the mice. This level of exposure to tamoxifen has previously been shown to produce a high incidence of liver tumours in rats (5,7). Mice were fed continuously and dietary intake of control and tamoxifen diet was constantly monitored by supplying the diet in standard open topped feeding containers that were weighed initially, then reweighed after 3 days to calculate the diet consumed by a group of three mice. Diet was continuously monitored from the start of the experiment for the first 2 months and then at monthly intervals until 9 months and then every 2 months until the end of the study. Clinical examinations for ill health were carried out on the animals twice daily and they were weighed weekly. At 3, 6 and 9 months, groups of five tamoxifen-treated and five control mice of each strain were killed in a rising concentration of CO₂. The numbers of C57BL/6 remaining at 12 months were 27 (27/33) treated and five control mice of each strain were killed in a rising concentration of CO₂. The numbers of DBA/2 mice remaining at 12 months were 26 (26/33) controls and 24 (24/33) tamoxifen-treated. The remaining C57BL/6 and DBA/2 mice were killed at 12 months because of the development of kyphosis. Kyphosis was manifested as an increase in the convex shape of the curvature of the thoracic spine, to give a hunchback appearance. At 12 months 26 tamoxifen treated B6C3F1 mice remained. Eleven out of 26 mice were taken off the tamoxifen diet, returned to the basal diet and administered 1000 p.p.m. phenobarbital (PB) in their drinking water until the end of the study (24 months). A similar proportion of the controls were administered PB in the same manner. PB was administered as a promoter of initiated hepatocytes, in an attempt to increase the incidence of liver tumours that might develop due to the initiating action of tamoxifen. The remaining B6C3F1 mice (15/26) continued the tamoxifen or basal diet until 24 months, at which time the study was terminated. Livers were removed, DNA extracted and subjected to 3P- and post-labelling as described below.

**Short-term study.** Female DBA/2, C57BL/6 and B6C3F1 mice (20–25 g), at three animals/group, were administered tamoxifen by daily oral gavage for 4 days at 40, 80 or 120 mg/kg dissolved in tricaprylin (0.1 ml) by gavage, daily for 4 days. Animals were killed 24 h after the last dose and at the times indicated, the livers were removed, DNA extracted and subjected to 3P- and post-labelling as described below.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Treatment time (months)</th>
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<tr>
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<tr>
<td>B6C3F1</td>
<td>Control</td>
<td>3 6 9 12</td>
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<tr>
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<td>Tam</td>
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<tr>
<td></td>
<td>Tam</td>
<td>1.2 ± 0.2 0.7 ± 0.1 1.8 ± 0.5 1.5 ± 0.2</td>
</tr>
<tr>
<td>DBA/2</td>
<td>Control</td>
<td>3.6 ± 0.3 0.7 ± 0.1 1.8 ± 0.5 1.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Tam</td>
<td>2.6 ± 0.2* 0.6 ± 0.3 2.0 ± 0.6 0.9 ± 0.2</td>
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PCNA labelling index expressed as stained cells per 10³ cells counted. A minimum of 4000 cells examined per animal. Results represent the mean ± SE from five animals. *Significantly reduced at the 5% level.

Newcastle-upon-Tyne, UK) followed by a rabbit anti-mouse IgG2a peroxidase conjugated antibody (1:50 dilution, Serotec, Oxford, UK). All procedures were carried out at room temperature. PCNA expressing cells were visualised using 3,3′-diaminobenzidine/H₂O₂ substrate. Sections were lightly counterstained with haematoxylin. Sections of duodenum, processed at the same time, served as positive controls. At least 4000 nuclei were examined on each section to derive the PCNA labelling index per 1000 nuclei examined.

**Determination of tamoxifen and its metabolites in liver and serum following 4 days oral dosing.**

Tamoxifen and its metabolites were estimated in protein-free extracts of serum or liver by reverse phase high performance liquid chromatography (HPLC) as described previously (13), using three animals from each group.

**DNA isolation and 32P-postlabelling of DNA adducts**

DNA was isolated by the method of Gupta (14) and subjected to 32P-postlabelling as described previously (3). In each experiment, liver DNA from a rat exposed to dietary tamoxifen for 3 months (~35 mg/kg) was also analysed and used as a positive control. Treatment of rats has been described previously (7). Except where indicated, three animals were analysed from each group, and at least three determinations were performed on each liver DNA sample.

**AMS analysis of mouse liver nucleotides**

Following enzymatic digestion of the DNA as described for the post-labelling procedure, nucleotides were subjected to reverse phase HPLC using a 25×0.4 cm C18 Tephisphere 5 μm column (HPLC Technology Ltd, UK) with a gradient solvent system of 20 mM ammonium acetate, adjusted to pH 3.0 with glacial acetic acid–methanol (0–40 min, 0–30% methanol; 40–60 min, 30–100% methanol; 100% methanol for 80 min) at a flow rate of 1 ml/min. UV absorbance was determined at 254 nm. Fractions (individual nucleotide peaks or subsequent 10-min fractions) were collected from the detector outlet, concentrated to dryness and converted to graphite for measurement by AMS (15).

**Statistical analysis**

The 3P-postlabelling results are expressed as relative adduct labelling (RAL), this assumes that normal and adducted nucleotides are labelled to an equal extent. The 32P-postlabelling data were analysed by the analysis of variance following a logarithmic transformation of the raw data, though the means presented show the untransformed values. The liver tumour incidence data was analysed using the Fischer exact test for differences between tamoxifen fed mice and control mice.

**Results**

**Liver pathology following long-term dietary administration**

In no strain of mouse studied was there an increase in the absolute liver weights at 3-, 6-, 9- or 12-month sacrifices relative to their respective control groups (data not shown) and the hepatocyte PCNA labelling indices were not increased (Table I). There was a significant decrease in the labelling index of the treated DBA/2 mice over the controls at 3 months; in no strain of mouse studied was there an increase in the DNA adduct half-life study. Female C57BL/6 mice (20–25 g), at three animals/group, were administered tamoxifen by daily oral gavage for 4 days after the last dose, the livers were removed, DNA extracted and subjected to 3P-postlabelling as described below.

**Tissue preparation.** From the long-term study, sections of all major lobes of the liver were fixed in 10% formaldehyde in buffered saline for routine histopathological examination and in Carnoy’s fluid for determination of the PCNA labelling index.

**Histopathological examination**

Sections (5 μm) were prepared from paraffin was embedded liver tissue. Representative sections of the left lateral, median, posterior and caudate lobes were included on slides prepared and stained with haematoxylin and eosin for histopathological examination.

**Liver cell replicative DNA synthesis assessed by proliferating cell nuclear antigen (PCNA) expression**

Paraffin sections (5 μm) from Carnoy’s-fixed liver were rehydrated. A monoclonal mouse anti-PCNA antibody was used (1:25 dilution, Novocastra, 2210
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24-month time points (data not shown). Altered hepatic foci were not found in the livers of mice prior to the formation of the three adenomas found in tamoxifen-treated B6C3F1 mice. None of the mouse strains developed hepatocellular carcinomas following tamoxifen treatment alone or after promotion with PB. The number and distribution of tumours in the B6C3F1 mice, which were killed after 24 months, did not demonstrate a statistically significant effect of tamoxifen treatment on tumour incidence at any site, including the liver, although a total of three liver adenomas were found in tamoxifen-treated animals as compared with none in the controls (Table II).

Hepatic concentration and serum levels of tamoxifen and its metabolites in three mouse strains following 4 days oral dosing

All three strains of mice showed non-linear accumulation of tamoxifen and metabolites in the liver with increasing dose of tamoxifen (Figure 1). In C57BL/6 and B6C3F1 mice, the concentration of N-desmethyltamoxifen and 4-hydroxytamoxifen was similar to that of tamoxifen. The relatively lower concentration of tamoxifen in the livers of DBA/2 mice was accompanied by a relatively higher 4-hydroxytamoxifen concentration. Serum levels of tamoxifen were 61 ± 3, 78 ± 5 and 135 ± 13 ng/ml (mean ± SE, n = 3) for DBA/2, C57BL/6 and B6C3F1 mice respectively.

Concentration of tamoxifen and its metabolites in livers of mice following long-term dietary administration

Although the dietary intake of tamoxifen in the DBA/2 strain was ~70% of the other two strains, concentrations of tamoxifen in the livers of DBA/2 mice after 3 months dietary exposure was 1.41 ± 0.17 nmol/g (mean ± SE, n = 4), which was similar to those of the C57BL/6 and B6C3F1 strains (1.64 ± 0.17 and 1.2 ± 0.33 nmol/g respectively). All three strains showed a similar pattern of metabolite accumulation. 4-Hydroxytamoxifen concentrations were 5.59 ± 0.31, 4.28 ± 0.59 and 3.0 ± 0.17 nmol/g liver in C57BL/6, DBA/2 and B6C3F1 strains respectively, while N-desmethyltamoxifen levels were 1.23 ± 0.23, 1.13 ± 0.16 and 0.64 ± 0.05 nmol/g liver respectively in the three strains. Serum levels this time were below the limits of detection (<0.03 nmol/ml). After 6 months of administration, levels of tamoxifen in the livers of dosed mice were 3.70 ± 0.90, 8.64 ± 1.01 and 9.90 ± 0.60 nmol/g for C57BL/6, DBA/2 and B6C3F1 strains respectively.

32P-Postlabelled adducts in the livers of mice

Following oral administration of tamoxifen for 4 days, one distinctive group of 32P-labelled adducts was observed, which was composed of at least two major products. This was observed in all three tamoxifen-treated mouse strains and was located near the top centre of the TLC plate (Figure 2). As shown in Figure 3, these major adducts increased significantly in all strains in a dose-dependent manner (40–120 mg/kg). The DBA/2 strain had a significantly higher average adduct level than the other two strains (RAL×106 was 31 in B6C3F1 and C57BL/6 strains, compared with 62 in the DBA/2 strain, P = 0.03). Following 3 months dietary tamoxifen administration, the major adducts were observed in treated liver of all three strains (Figures 2 and 4). There were no significant differences between the adduct levels observed in the strains (P > 0.05). By 6 and 12 months these adducts were reduced and had decreased to background levels in B6C3F1 mice at...
control animals were low at all time points (RAL 24 months (Figures 2 and 4). Adducts detected in livers from DNA extracted and subjected to $^{32}$P-post-labelling. Three mice were used at each dose level. Results represent mean ± SD.

![Graph](image)

**Fig. 3.** Dependence on dose of tamoxifen in the major DNA adducts detected in livers of mice in the short-term study. Female DBA/2, C57BL/6 and B6C3F1 mice were dosed orally with tamoxifen for four days at 40, 80 or 120 mg/kg. Animals were killed 24 h after the last dose, the livers were removed, DNA was extracted and subjected to $^{32}$P-post-labelling. Individual bars represent different mouse livers. Adduct levels expressed as the log of the mean were linear with dose. Two mice were used at each dose level. Results represent mean ± SD.

![Graph](image)

**Fig. 4.** Dependence on time of exposure to tamoxifen on the accumulation of the major DNA adducts detected in livers of mice. Female DBA/2, C57BL/6 and B6C3F1 mice were fed tamoxifen in the diet to give a daily dose of 100–120 mg/kg. After 8 weeks the diet was reduced to one-third. DBA/2 and C57BL/6 mice were killed at 3 and 6 months. B6C3F1 were killed at 3, 6, 12 and 24 months. Studies using C57BL/6 and DBA/2 mice were terminated after 6 months due to kyphosis. Livers were removed, DNA was extracted and subjected to $^{32}$P-post-labelling. Three mice were used at each time point except at 6 months when two mice were used. Results are mean ± SD.

24 months (Figures 2 and 4). Adducts detected in livers from control animals were low at all time points (RALx$10^6 < 6$).

**HPLC and AMS analysis of mouse liver nucleotides**

To demonstrate the major adducts detected in mouse liver were a result of tamoxifen or a metabolite of tamoxifen binding with DNA, mice were treated with $[^{14}$C]tamoxifen, and DNA was digested to nucleotides and separated by HPLC. Fractions were collected as described in Materials and methods and analysed for their $^{14}$C content by AMS (Figure 5). There was no increase in $^{14}$C-radiolabel in the normal nucleotide fractions, demonstrating the absence of incorporation of $^{14}$C-label into the normal nucleotide pool. In the nucleotide fractions, no peak attributed to uracil was detected, demonstrating the absence of RNA contamination. Figure 5 shows that the majority of the $^{14}$C-radiolabel elutes between 51 and 60 min. There was no evidence of unchanged tamoxifen, which elutes much later at ~72 min.

**Half-life of tamoxifen DNA adducts in mouse liver**

The major tamoxifen DNA adducts disappeared from the liver DNA of C57BL/6 mice following treatment with 120 mg/kg of tamoxifen for 4 days in a bi-phasic manner, with a rapid initial loss within 3 days followed by a slower removal over several weeks (Figure 6).

**Discussion**

A lifetime feeding study (2 years) with tamoxifen was achieved for the first time in the B6C3F1 strain of mouse because of its resistance to the kyphosis inducing effect of tamoxifen. Kyphosis occurred in the two other strains (C57BL/6 and DBA/2) and also in the original study, performed using Alderley Park (AP) mice, which were killed at 15 months (9). At present it is not understood why the B6C3F1 strain is resistant to the formation of skeletal abnormalities caused by tamoxifen. No hepatocellular carcinomas were observed in any of the strains, even in B6C3F1 mice after PB promotion.

**Pathological effects of tamoxifen on the livers of mice**

The livers of mice fed tamoxifen for up to 2 years did not show the steady increase in number of altered hepatic foci that is seen as a precursor to eventual tumour formation in rats administered tamoxifen (7). Even when PB was used to promote the formation of liver tumours, there was no significant increase in the incidence of mouse liver tumours, in contrast to the formation of liver tumours promoted in rats with PB (3). There was also a notable failure of tamoxifen to increase the PCNA hepatocyte labelling index in mice given tamoxifen for up to 2 years. In fact the liver weights of tamoxifen fed mice were significantly lower than the respective controls at sacrifice times, and there was a decrease in the PCNA labelling index, compared with control values for two of the mouse strains at two sacrifice time points (C57BL/6 at 6 months and DBA/2 at 3 months, see Table I).

**Hepatic concentrations of tamoxifen and its metabolites**

Determination of tamoxifen in the livers of mice dosed orally for 4 days showed a non-linear accumulation in all three strains (Figure 1). Concentrations of tamoxifen in mouse liver were 50- to 100-fold lower relative to rats given the same dose (7) and this is consistent with the rapid clearance of tamoxifen in mice, relative to other species (16). Qualitatively, the pattern of metabolism of tamoxifen in mice and rats are similar (17). Quantitatively, treated mice accumulated relatively high levels of 4-hydroxytamoxifen in the liver (Figure 1), whereas in rats, N-desmethyltamoxifen was the major hepatic metabolite (7). These quantitative differences in metabolism of tamoxifen to polar hydroxylated derivatives in the mouse may facilitate the elimination of this drug as Phase II conjugates (18,19). No attempt was made to measure α-hydroxytamoxifen in this study.

**DNA adducts in the livers of mice**

Following $^{32}$P-postlabelling of liver DNA of all mouse strains treated orally with tamoxifen for 4 days, one distinctive group of $^{32}$P-labelled adducts composed of at least two major components was observed at the top centre of the TLC plate, and was absent from controls. In similarly dosed Fischer rats...
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Fig. 5. Irreversible covalent binding of [14C]tamoxifen to hepatic non-polar DNA adducts. DNA extracted from the livers of mice treated with a single dose of [14C]tamoxifen (120 mg/kg) and killed 24 h later was subjected to enzymatic hydrolysis followed by HPLC as described in the Materials and methods. (A) The typical trace of the relative UV absorption (254 nm) from a tamoxifen treated mouse. Fractions collected from the detector were concentrated to dryness under vacuum and assayed for 14C content by AMS. (B) The radioactive profile from fractions collected from (A). Solid and hatched bars represent the DNA hydrolysates from control and dosed mice respectively.

Fig. 6. Loss of tamoxifen–DNA adducts in mouse liver following cessation of dosing. Female C57BL/6 mice were dosed orally with tamoxifen (120 mg/kg) daily for 4 days. At 24 h after the last dose and at 3, 7, 14, 21 and 35 days thereafter, animals were killed and the livers removed. DNA was extracted and subjected to 32P-post-labelling. Three mice were used for each time point except for 14 days when two mice were used. Results represent mean ± SD. The dotted line represents control levels that had a RAL of 3.6 ± 1.0×10^3.

Similar to the results observed in rats (20), adducts were observed in the mice treated orally with tamoxifen (45 mg/kg) for 4 days, levels of liver DNA 32P-postlabelled adducts were ~170 adducts/10^8 nucleotides (10), which is 10-fold higher than seen in mice (Figure 3). The group of adducts observed in the mice had similar Rf values to the major DNA adducts observed in rat liver following tamoxifen treatment (Figure 2). The structural relationship between the adducts seen in the mouse and the rat is presently unclear. AMS analysis of HPLC separated nucleotides from mouse liver shows that the majority of the 14C-radiolabel elutes between 51 and 60 min (63.1% total 14C). A similar chromatogram is observed in rats following a similar dosing regime (20), and the 32P-postlabelled products of the fraction that is obtained between 51 and 60 min have a similar Rf value on TLC plates as the major rat adducts, further confirming that adducts in this HPLC fraction are tamoxifen derived.

During long-term exposure to tamoxifen, hepatic DNA adducts were observed in all mouse strains at 3 months, but after this time point DNA adducts did not accumulate but decreased to control levels by 24 months. The reason that the adducts disappear while dosing is continuing, is presently unknown, but there are several possibilities. The first hypothesis is that levels of tamoxifen and its metabolites are low in the liver. Our results show that tamoxifen is rapidly cleared from the liver of mice following dosing, which results in low hepatic concentrations of this drug and its metabolites. This would prevent the formation of large numbers of adducts in the liver and would stop tamoxifen being stored in lipid depots of cells, for future use. In certain strains of rats given tamoxifen for 6 months, there is evidence of a decrease in the levels of tamoxifen in the liver (7) associated with an increase in P-glycoprotein expression (21). It is suggested that P-glycoprotein plays a causal role in the disappearance of the tamoxifen DNA adducts by progressively depleting the supply of drug for
intracellular metabolic activation. Thereby the rate of adduct formation would fall behind the rate of adduct removal. Tamoxifen requires metabolic activation to a DNA binding intermediate, and several research groups have identified possible reactive pathways. These include sulphation of \( \alpha \)-hydroxytamoxifen to give \( \alpha \)-sulphate cis tamoxifen (22,23). The model, \( \alpha \)-acetoxytamoxifen, has been reacted in vitro with DNA to give products chromatographically identical to DNA adducts found in treated rat liver. The major in vitro adduct has been identified as a deoxyguanosine adduct in which the \( \alpha \) position of tamoxifen is linked covalently to the exocyclic amino group of deoxyguanosine (22,23,24). The minor adducts are the trans-cis isomer of the guanosine adduct and a tamoxifen–deoxyadenosine adduct (24). Other proposed reactive intermediates include an aromatic epoxide (17) or conversion of 4-hydroxytamoxifen to a quinone methide (25).

The second hypothesis explaining the loss of DNA adducts during long-term tamoxifen administration is an efficient DNA repair mechanism of adducts in mouse liver, eliminating them when they appear. Our results show that the half-life of the tamoxifen DNA adducts in mouse liver of a few days is very much shorter than the several months half-life in rat liver DNA (3). This suggests especially effective DNA repair mechanisms in murine liver, since if it was only a question of chemical instability of the adducts, we would expect to see similar half-lives in rat and mouse livers. Others have also found shorter half-lives of certain DNA adducts in mice compared with rats. For example, liver 7-(2-hydroxyethyl) guanine DNA adducts have half-lives of 1 and 3.9 days in mouse and rats respectively (26).

Comparison with rat and human data

Our studies indicate that there appears to be a causal relationship between the level of tamoxifen induced DNA adducts in the liver and the eventual development of tumours. After 3 months of dietary administration of tamoxifen, our results show that adduct levels in Wistar rats are \( \approx 2500 \) adducts/10\(^8\) nucleotides (7), compared with levels of \( \approx 70 \) in similarly treated mice. In rats, adduct levels continue to increase and then plateau by 6 months, whereas in mice, levels decrease and can no longer be detected at 24 months.

Numbers of DNA adducts are not necessarily related to the rate of metabolism of tamoxifen. Presently it is unknown which adducts in rat liver are fixed as mutations, promoted and subsequently expressed as tumours. The adducts responsible may be absent or in too low a concentration in mouse liver to initiate the events resulting in final tumorigenic outcome. Tamoxifen causes endometrial tumours in women (27), however tamoxifen induced DNA adducts are extremely low or absent in the human liver (28) and endometrium (29,30). The mutagenic adduct(s) in tamoxifen-treated rats and the threshold level of this adduct(s) for tumour outcome needs to be identified.

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