Effects of Chronic Administration of Tamoxifen and Toremifene on DNA Adducts in Rat Liver, Kidney, and Uterus

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Abstract

To assess the effects of chronic administration of tamoxifen (TAM) and toremifene (TOR) on genetic damage related to carcinogenesis, we measured DNA adduct formation by 32P-postlabeling in liver, kidney, and uterus of Fischer rats given TAM or TOR in the diet for 18 months. TAM induced high levels of DNA adducts in the liver in a dose-dependent manner. The total adduct levels were 3000 ± 870 and 6100 ± 1500 adducts per 10^9 nucleotides for the 250- and 500-ppm groups, respectively. TOR induced a dose-dependent level of adducts that was lower than that observed for TAM. The total hepatic adduct level was 70 ± 5, 130 ± 20, and 70 ± 20 for 250, 500, and 750 ppm TOR, respectively. Both TAM and TOR induced a low level of adducts in the kidneys, and TOR significantly enhanced endogenous DNA adduct formation. The total adduct level was 480 ± 140, 420 ± 210, and 680 ± 80 adducts per 10^9 nucleotides for control, 500 ppm TAM, and 500 ppm TOR, respectively. Although neither TAM nor TOR induced adducts in the uterus, TAM significantly enhanced endogenous DNA modifications in this tissue. The total uterine adduct level was 70 ± 30, 130 ± 50, and 70 ± 20 for control, 500 ppm TAM, and 500 ppm TOR, respectively. These observations demonstrate a correlation between DNA adduct formation and carcinogenicity for these compounds. The effectiveness of TOR and TAM in increasing endogenous DNA adducts indicates that a mechanism other than direct DNA damage may also be involved in their carcinogenicity.

Introduction

Tamoxifen (TAM) has been used widely as an adjuvant therapy for the treatment of breast cancer and is presently undergoing clinical trials as a chemopreventive agent in women at high risk of developing this disease (1). The recent findings that TAM induces liver cancer in rats (2, 3) and increases the incidence of endometrial cancer in exposed women (4–6) have aroused controversy over the use of TAM in large-scale prophylactic trials in healthy women (7). These concerns have led to an interest in the development of alternative drugs that have a lower toxicity upon chronic administration. TOR, a structural analogue of TAM, has a pharmacological profile similar to that of TAM (21), the effect of this structurally and pharmacologically antiestrogenic compound on DNA damage in various tissues should also be evaluated.

One of our laboratories has previously reported the results of tumor-promoting activity of TAM and TOR in a two-stage hepatocarcinogenesis rat model after 18 months of dietary administration (22). The current report presents the results of the analysis of DNA adducts in the liver, kidney, and uterus of rats exposed to TAM or TOR for 18 months. Information generated from the long-term study may be more relevant to human risk assessment than that from acute studies.

Materials and Methods

Materials. TAM was obtained from Sigma Chemical Co. (St Louis, MO). TOR was a gift from Dr. Lauri Kangas (Farmos Pharmaceutical Co., Turku, Finland). The diets were formulated and prepared by Teklad Test Diets (Madison, WI). Enzymes and [γ-32P]ATP used for 32P-postlabeling assay are as described previously (22). PEI-cellulose plates (Polygram CEL 300 PEI made by Macherey-Nagel) were purchased from Bodman (Asdon, PA).

Animal Treatment. Tissue samples used for DNA adduct analysis were obtained from a previous study of tumor-promoting activity of TAM and TOR by measuring the altered hepatic foci following an initiation-promotion protocol in a rat liver model (22). Briefly, female Fischer rats of 130 ± 10 g were obtained from Harlan Sprague Dawley (Indianapolis, IN) and maintained on a Teklad mouse/rat 4% fat diet in an environment of 12-h light and dark cycles. Rats were housed three per cage and provided with food and water ad libitum. After 1 week of acclimatization in the laboratory, all of the rats were subjected to a 70% partial hepatectomy. Twenty-four h after surgery, all of the rats used in this study were administered a single dose of the solvent, trioctanoin. Two weeks after the surgery, rats were placed on a basal diet alone or containing TAM (250 and 500 ppm diet) or TOR (250, 500, and 750 ppm diet). Rats were maintained on these diets for 18 months, with a month defined as 28 days. Liver, kidney, and uterus were obtained when the rats were killed, and tissue samples were frozen immediately and kept at −80°C until DNA isolation.

DNA Adduct Analysis. DNA adduct analyses were performed using the nuclease P1-enhanced version of the 32P-postlabeling procedure (19). The DNA samples were digested with 10 units of nuclease P1 on ice for 30 min. After digestion, DNA was dialyzed overnight against 0.02 M sodium phosphate, pH 7.2, and 0.1 M sodium chloride. DNA samples were then dried in a SpeedVac concentrator and resuspended at a concentration of 50 μg/μl in 0.02 M sodium phosphate, pH 7.2, and 0.1 M sodium chloride. DNA was then subjected to 32P-postlabeling using [γ-32P]ATP and the following conditions: 0.4 M sodium phosphate, pH 5.75, 0.6 M sodium chloride, 1.5 M sodium acetate, and 2.5 U of polynucleotide kinase in a final volume of 100 μl. Samples were incubated at 37°C for 20 min, and the reaction was stopped by boiling for 5 min. DNA samples were then applied to a PEI-cellulose plate (Polygram CEL 300 PEI) and developed with 2.3 M sodium phosphate, pH 5.75, at 80°C.

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3 The abbreviations used are: TAM, tamoxifen; TOR, toremifene; RAL, relative adduct labeling; DEN, diethylnitrosamine.

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Tris, 5.0 M urea, pH 8.0, for D4. The central maps were developed in 60% dimensional chromatography were: 60% LFU, i.e., 2.5 M lithium formate, 4.5 the lower and central part of the D1 chromatogram. Solvents used for two in all three individual rats. Quantitative data are given in Table 1.

Numbered spots are major adducts consistently detected for 18 months. A and B, control; C, TAM; D, bR. Film exposures were at —80°C for 1 h for A and C and 15 h for B and D. Numbered spots are major adducts consistently detected for 18 months induced a high level of DNA adduct formation in the liver (Table 1). Although the level of total adducts was only 2% of that in TAM-treated rats (Table 1), chronic administration of TOR also induced DNA adduct formation in the liver. At least six DNA adducts were detected in the liver of TOR-exposed rats (Fig. 1). The most prominent effect of TOR was seen in the 500-ppm samples, which contained a significantly higher level of adducts (130 ± 20 \( \times 10^3 \)) than the other two dose groups (70 ± 5 for 250 ppm and 70 ± 20 for 750 ppm). At the 250- and 500-ppm dose levels of TOR, a dose-dependent increase in adduct formation was observed (Table 1). However, at the highest tested dose, i.e., 750 ppm, the adduct level was lower than that in the middle dose group (Table 1).

**DNA Adducts in the Kidney.** Most adduct spots detected in the kidney of TAM-treated rats were also present in the nontreated controls. These adducts have been described previously as indigenous DNA modifications, i.e., 1-compounds that are not directly a function of carcinogen exposure. Among the three tissues examined in this study, the kidney had the highest level of 1-compounds (Table 2). Rechromatographic analysis of the 3 lower spots and 3 central spots (as numbered in Fig. 2) that were common for both control and TAM-treated rats did not reveal any additional TAM-adduct formation (data not shown). Although the total level of adducts detected in TAM-treated rats (420 ± 210) was not significantly different from that in controls (480 ± 140), the presence of a TAM-generated adduct with relatively strong intensity was detected on the lower maps of the kidney (Fig. 2).

<table>
<thead>
<tr>
<th>Spot</th>
<th>TAM250</th>
<th>TAM500</th>
<th>TOR250</th>
<th>TOR500</th>
<th>TOR750</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>200 ± 80</td>
<td>770 ± 360</td>
<td>10 ± 2</td>
<td>13 ± 3</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>L2</td>
<td>1600 ± 750</td>
<td>2800 ± 680</td>
<td>5 ± 2</td>
<td>10 ± 3</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>L3</td>
<td>210 ± 1</td>
<td>400 ± 90</td>
<td>6 ± 3</td>
<td>13 ± 6</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>L4</td>
<td>510 ± 20</td>
<td>1200 ± 310</td>
<td>8 ± 2</td>
<td>14 ± 4</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>L5</td>
<td>50 ± 4</td>
<td>130 ± 50</td>
<td>5 ± 1</td>
<td>14 ± 10</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>L6</td>
<td>70 ± 5</td>
<td>90 ± 40</td>
<td>5 ± 4</td>
<td>5 ± 3</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>L1-6</td>
<td>2800 ± 830</td>
<td>5700 ± 1400</td>
<td>40 ± 5</td>
<td>70 ± 3</td>
<td>38 ± 10</td>
</tr>
<tr>
<td>Other</td>
<td>240 ± 40</td>
<td>420 ± 80</td>
<td>30 ± 10</td>
<td>60 ± 20</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>Total</td>
<td>3000 ± 870</td>
<td>6100 ± 1500</td>
<td>70 ± 5</td>
<td>130 ± 20</td>
<td>67 ± 20</td>
</tr>
</tbody>
</table>

* 250 and other numbers in this row represent dose levels of TAM or TOR as ppm in the diet.

![Figure 1](link) DNA adduct patterns in liver DNA of rats exposed to 500 ppm TAM or TOR for 18 months. A and B, control; C, TAM; D, TOR. Film exposures were at —80°C for 1 h for A and C and 15 h for B and D. Numbered spots are major adducts consistently detected in all three individual rats. Quantitative data are given in Table 1.

**Results**

**DNA Adducts in the Liver.** In concert with findings reported previously in studies of acute administration, TAM given chronically for 18 months induced a high level of DNA adduct formation in the liver in a dose-dependent manner (Table 1). Six major adducts (spots 1–6, Fig. 1) were consistently detected in all TAM-exposed livers. The strongest adduct was spot 2, with an average RAL \( \times 10^3 \) value of 1600 ± 740 and 2800 ± 700 in rats exposed to 250 and 500 ppm TAM, respectively, which accounted for 45–53% of the total adducts in the liver (Table 1).

### Table 2 Chronic antiestrogen treatment results in DNA adducts in the rat kidney and uterus

<table>
<thead>
<tr>
<th>Spot</th>
<th>CON</th>
<th>TAM</th>
<th>TOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>10 ± 4</td>
<td>30 ± 6</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>L2</td>
<td>10 ± 3</td>
<td>20 ± 4</td>
<td>50 ± 5</td>
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<tr>
<td>L3</td>
<td>70 ± 6</td>
<td>60 ± 8</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>L_{total}</td>
<td>130 ± 20</td>
<td>140 ± 30</td>
<td>150 ± 30</td>
</tr>
<tr>
<td>C1</td>
<td>210 ± 70</td>
<td>140 ± 150</td>
<td>290 ± 50</td>
</tr>
<tr>
<td>C2</td>
<td>120 ± 40</td>
<td>100 ± 40</td>
<td>170 ± 30</td>
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<tr>
<td>C3</td>
<td>20 ± 5</td>
<td>30 ± 20</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>C_{total}</td>
<td>350 ± 120</td>
<td>280 ± 200</td>
<td>540 ± 80</td>
</tr>
<tr>
<td>Total</td>
<td>480 ± 140</td>
<td>420 ± 210</td>
<td>690 ± 80</td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>7 ± 3</td>
<td>9 ± 2</td>
<td>8 ± 5</td>
</tr>
<tr>
<td>L_{total}</td>
<td>30 ± 20</td>
<td>40 ± 20</td>
<td>30 ± 20</td>
</tr>
<tr>
<td>C1</td>
<td>10 ± 4</td>
<td>3 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>C2</td>
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<td>10 ± 3</td>
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<td>10 ± 5</td>
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<td>20 ± 15</td>
</tr>
<tr>
<td>C_{total}</td>
<td>40 ± 10</td>
<td>80 ± 30</td>
<td>40 ± 20</td>
</tr>
<tr>
<td>Total</td>
<td>70 ± 30</td>
<td>130 ± 50</td>
<td>70 ± 20</td>
</tr>
</tbody>
</table>

* Kidney and uterus samples were from female Fischer rats maintained on a basal diet alone (CON) or this diet containing the antiestrogen tamoxifen (TAM) or toremifene (TOR) at 500 ppm.

* \( I_{total} \) or \( C_{total} \) represents the level of total adducts determined by \(^3\)P-postlabeling on the Lower or Central adduct maps, respectively.
on DNA modifications in the uterus (Fig. 2; Table 2). The total hepatic DNA adducts induced by TOR were only 2% of the level induced by TAM, supporting an association between carcinogenicity and DNA adduct formation.

Although TOR at the highest dose (750 ppm) increased the incidence of hepatocellular carcinoma in the DEN-initiated groups to a level one-third that observed with TAM administration to DEN-initiated rats, TOR alone did not induce any malignant hepatic neoplasms (22). The differences in carcinogenic action of TAM and TOR may not be ascribed to their estrogenic activities but could be associated with the relatively lower genotoxicity of TOR than TAM (20, 23). TOR-induced chromosome damage and DNA adduct formation in rat livers have been reported in previous studies (12, 24). Results of the current study further demonstrate that chronic administration of TOR to Sprague Dawley rats induced DNA adduct formation in the liver. At the same dose level, the total hepatic DNA adducts induced by TOR were only 2% of the level induced by TAM, supporting an association between carcinogenicity and DNA adduct formation.

Both TAM and TOR increased the incidence of renal cell carcinomas in previously DEN-initiated rats but not in non-initiated rats (22). Although both TAM and TOR induced DNA adduct formation in the kidney, the levels of these adducts were low and did not result in a significant increase in the total adduct levels between the exposed and untreated control rats. However, the possibility that chronic accumulation of these low-level adducts may contribute to the tumor-promoting activities of these compounds cannot be excluded. On the other hand, the kidney contains a high level of endogenous DNA modifications, and that the level decreased to 440/10⁸ nucleotides 3 months after withdrawal of TAM (18). Because TAM was not detected in the liver of rats 3 months after dosing was discontinued, these findings indicate that TAM-induced DNA adducts are relatively persistent. With continuous administration of TAM for 18 months, the adducts detected at the end of the study could be a consequence of the accumulation of persistent DNA adducts.

Discussion

The major findings of this study are that chronic TAM administration induced abundant DNA adducts in rat liver and that chronic TOR administration also induced DNA adducts in the rat liver, albeit at a lower level than TAM. In addition, both TAM and TOR induced a low level of adducts in the kidney but not in the uterus. These observations support a correlation between DNA adduct formation and the subsequent formation of neoplasms in TAM- and TOR-exposed animals.

It has been reported previously that after 18 months of administration, the highest concentration of TAM (500 ppm) increased the incidence of malignant hepatic neoplasms in Fischer rats (22). Another study also found that even in the absence of a promoting agent, 3 months of exposure to TAM (420 ppm) followed by a control diet for 20 months in Wistar rats was sufficient to cause liver tumor formation (18). The carcinogenicity of TAM may be directly attributable to a genotoxic activity in rat liver. In the current study, chronic administration of TAM induced a dose-dependent, high level of DNA adduct formation in the liver, the major target tissue of TAM-induced carcinogenesis in rats. The adducts detected at the end of the 18-month exposure could reflect a balance between adduct formation and removal during the long-term exposure. A recent study has reported that dietary administration of TAM (420 ppm) to Wistar-AP female rats for 3 months induced DNA adducts at the level of 720/10⁸ nucleotides, and that the level decreased to 440/10⁸ nucleotides 3 months after withdrawal of TAM (18). Because TAM was not detected in the liver of rats 3 months after dosing was discontinued, these findings indicate that TAM-induced DNA adducts are relatively persistent. With continuous administration of TAM for 18 months, the adducts detected at the end of the study could be a consequence of the accumulation of persistent DNA adducts.

Chronic TOR treatment induced several DNA adducts in the rat kidney, both on the lower map and one adduct on the central map in the kidney (Fig. 2). The intensity of each adduct was, however, relatively low. In addition, TOR significantly enhanced the level of endogenous polar I-compounds in the kidney relative to either TAM or control (Table 2). The RAL × 10⁸ values of the total central spots were 350 ± 120 and 540 ± 80 for control and TOR groups, respectively. As a consequence of this enhancement, the total adduct level was also significantly higher in TOR-exposed rats (680 ± 80) than that in control (480 ± 140) and TAM-exposed rats (40 ± 20). The numbered spots (I-compounds) in Fig. 2 that were common for control and TOR rats were also analyzed by rechromatography, and no further separation was detected in the solvent systems used.

DNA Adducts in the Uterus. Neither TAM nor TOR induced any DNA adducts in the uterus (Fig. 2). However, TAM significantly enhanced the formation of an endogenous adduct, spot 3, on the central maps (RAL × 10⁸ values of 60 ± 30) compared with controls (10 ± 5). This enhancement accounted for the significantly higher level of total adducts in the uterus of TAM-treated rats (130 ± 50) than in controls (70 ± 30). TOR did not show any significant effect on DNA modifications in the uterus (Fig. 2; Table 2).

![Fig. 2. 32P-labeled DNA adducts in kidney (upper panels) and uterus (lower panels) of rats treated with 500 ppm TAM or TOR for 18 months. C, central maps; L, lower maps. CON, controls. Film exposure was at —80°C for 1 h (C maps) and 5 h (L maps) for the kidney and 16 h for the uterus maps. Numbered spots were analyzed by rechromatography. TAM induced one bulky adduct and TOR induced several weak spots in the kidney on the L maps as indicated by arrows. TAM and TOR did not induce any extra adducts, but TAM enhanced the intensity of spot 3 on the C maps of the uterus.](https://cancerres.aacrjournals.org/content/57/23/5125/F2.large.jpg)
compared with controls. Certain of these unidentified DNA derivatives may play a role as premutagenic lesions, hence contributing to carcinogenesis.

Epidemiological evidence suggests that in women treated with TAM, neoplasms of the endometrium may be more important than carcinogenesis. Certain of these unidentified DNA derivatives may play a role as premutagenic lesions, hence contributing to carcinogenesis. TAM, however, enhanced the polar I-compounds in the uterus when rats received multiple doses of TAM (17). In our long-term study, neither TAM nor TOR induced DNA adduct formation in the uterus. These findings suggest that TAM and TOR may not be genotoxic or carcinogenic in the uterus of rats under the conditions of our study. TAM, however, enhanced the polar I-compounds in the uterus, which may be related to the decreased body weight gain (22) or to the modulation of microsomal enzymes (26) observed with TAM administration. These results suggest that, besides a direct DNA-damaging effect, TAM may additionally exert an indirect carcinogenic potential through enhancing the formation of endogenous DNA modifications. Further understanding of the metabolic differences in human and rat endometrium is required to better assess the carcinogenic risk that TAM poses to the human endometrium.

References
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