Mechanism of Lower Genotoxicity of Toremifene Compared with Tamoxifen


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ABSTRACT

An increased incidence of endometrial cancer has been reported in breast cancer patients taking tamoxifen (TAM) and in healthy women participating in the TAM chemoprevention trials. Because TAM-DNA adducts are mutagenic and detected in the endometrium of women treated with TAM, TAM adducts are suspected to initiate the development of endometrial cancer. Treatment with TAM has been known to promote hepatocarcinoma in rats, but toremifene (TOR), a chlorinated TAM analogue, did not. TAM adducts are primarily formed via sulfonation of the α-hydroxylated TAM metabolites. To explore the mechanism of the lower genotoxicity of TOR, the formation of DNA adducts induced by TOR metabolites was measured using 32P-postlabeling/high-performance liquid chromatography analysis and compared with that of TAM metabolites. When α-hydroxytoremifene was incubated with DNA, 3-phospho-α-deoxyguanosine 5'-phosphosulfate, and either rat or human hydroxysteroid sulfotransferase, the formation of DNA adducts was two orders of magnitude lower than that of α-hydroxytamoxifen. α-hydroxytoremifene was a poor substrate for rat and human hydroxysteroid sulfotransferases. In addition, the reactivity of α-acetoxytoremifene, a model activated form of TOR, with DNA was much lower than that of α-acetoxytamoxifen. Thus, TOR is likely to have lower genotoxicity than TAM. TOR may be a safer alternative by avoiding the development of endometrial cancer.

INTRODUCTION

TAM1 (the structure in Fig. 1) is widely used as a first-line therapy for breast cancer patients (1). A randomized clinical trial for healthy women at high risk of developing this disease, conducted by the National Surgical Adjuvant Breast and Bowel Project, showed that therapeutic doses of TAM reduced the risk of invasive breast cancer approximately 50% (2). Therefore, this drug was approved in 1998 by the Food and Drug Administration for use as a chemopreventive agent. Unfortunately, administration of TAM to breast cancer patients was associated with an increased risk of endometrial cancer (3–8). The increased incident of endometrial cancers was also observed at the TAM chemoprevention trial (2, 4). TAM is a potent hepatocarcinogen in rats (9–11) and produces a high level of TAM-DNA adducts in the liver of rats treated with this drug (12–15). TAM was associated with an increased risk of endometrial cancer (3–8). TAM is a potent hepatocarcinogen in rats (9–11) and produces a high level of TAM-DNA adducts in the liver of rats treated with this drug (12–15). TAM was associated with an increased risk of endometrial cancer (3–8).

TOR (Fig. 1), a chlorinated TAM derivative, has been used for breast cancer therapy in 27 countries, including in Finland since 1988, in Sweden since 1994, in Japan since 1995, and in the United States since 1998. TOR is metabolized by human liver microsomes, resulting in the formation of N-desmethyltoremifene, 4-hydroxytoremifene, and deaminohydroxytoremifene; the formation of N-desmethyltoremifene and deaminohydroxytoremifene are mediated by P450 1A and 3A4 enzymes (32). The N-desmethylated and 4-hydroxylated metabolites were detected in human blood (33) and mainly excreted in rat feces (34, 35). Small amounts of these metabolites are excreted in rat urine; α-OHTOR and α-hydroxy-N-desmethyltoremifene were also detected using mass spectroscopy (35). Unlike TAM, TOR produces only a small amount of DNA adducts in rat liver (11, 36, 37) and does not promote hepatocarcinoma in rats (11, 36).

In the present study, to investigate the mechanism of the lower genotoxicity of TOR, we synthesized α-OHTOR and α-acetoxyTOR as model activated forms of TOR. We determined whether α-OHTOR can be sulfonated by hydroxysteroid sulfotransferases and whether α-acetoxyTOR can react with DNA.

MATERIALS AND METHODS

Chemicals. [γ-32P]ATP (specific activity, 6000 Ci/mmol) was obtained from Amersham Pharmacia Biotech, Inc. (Piscataway, NJ). PEI-cellulose plates were purchased from Machery-Nagel (Dueren, Germany). Calf thymus DNA, proteinase K, potato apyrase, dG3P,2'-deoxyguanosine, dG,2'-deoxyguanosine 3'-monophosphate; HPLC, high-performance liquid chromatography; TOR, toremifene; RAL, raloxifene; α-OHTAM, α-hydroxytamoxifen; α-acetoxyTAM, α-acetoxytamoxifen; α-OHTOR, α-acetoxytoremifene; α-acetoxyTOR, α-acetoxytoremifene; AAP, adenosine 3',5'-diphosphate; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; HIST, human hydroxysteroid sulfotransferase (SULT2A1); STa, rat hydroxysteroid (alcohol) sulfotransferase a; tig, retention time; NMR, nuclear magnetic resonance; s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets.

TAM is metabolized in the liver of rodents and humans to α-OHTAM, N-desTAM, tamoxifen N-oxide, and 4-hydroxytamoxifen (17–19). Among these TAM metabolites, we found that α-OHTAM is sulfonated by hydroxysteroid sulfotransferases (20, 21) and reacts with the exocyclic amino group of guanine in DNA, resulting in the formation of two trans (fr-1 and fr-2) and two cis (fr-3 and fr-4) diastereoisomers of dG-N2-TAM (Fig. 2; Refs. 13 and 22). A mass-spectroscopy analysis (23) and 32P-postlabeling/HPLC analysis (24, 25) demonstrated that dG-N2-TAM and α-(N2-deoxyguanosinyl)-N-desmethyltamoxifen are major DNA adducts in the liver of rodents treated with TAM. These indicated that TAM-DNA adducts are primarily formed via sulfonation of α-hydroxylated TAM metabolites such as α-OHTAM and α-hydroxy-N-desmethyltamoxifen (Fig. 2). A high frequency of mutations was observed in the liver DNA of ALL acl transgenic rats treated with TAM (26). dG-N2-TAM adducts display a high miscoding and mutagenic potential and generate primarily G→T transversions in mammalian cells (27, 28). Significant level of dG-N2-TAM adducts have been detected in the endometrium of certain women treated with TAM (29, 30). Such TAM adducts, if not repaired (31), may cause mutations, leading to the development of endometrial cancers.

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3 The abbreviations used are: TAM, tamoxifen; dG, 2'-deoxyguanosine; dG,2'-deoxyguanosine 3'-monophosphate; HPLC, high-performance liquid chromatography; TOR, toremifene; RAL, raloxifene; α-OHTAM, α-hydroxytamoxifen; α-acetoxyTAM, α-acetoxytamoxifen; ddG-N2-TAM, α-(N2-deoxyguanosinyl)tamoxifen; N-desTAM, N-desmethyltamoxifen; PEP, polyethyleneimine; α-OHTOR, α-hydroxytoremifene; α-acetoxyTOR, α-acetoxytoremifene; AAP, adenosine 3',5'-diphosphate; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; HIST, human hydroxysteroid sulfotransferase (SULT2A1); STa, rat hydroxysteroid (alcohol) sulfotransferase a; tig, retention time; NMR, nuclear magnetic resonance; s, singlet; d, doublet; t, triplet; m, multiplet; dd, doublet of doublets.
a solution of (E)-1-bromo-2-[4-[2-(dimethylamino)ethoxy]phenyl]-1,2-diphenyl ethene (720 mg; 1.7 mmol) in dry tetrahydrofuran (12 ml) at -78°C under N2. Distilled chloroacetaldehyde (0.39 g; 5.1 mmol) in 5 ml of tetrahydrofuran was added 5 min later. The mixture was warmed to 0°C in 20 min, quenched with water (10 ml), extracted with ether (2 x 20 ml), dried over anhydrous sodium sulfate, and then concentrated. The residue was purified by column chromatography using silica gel (eluent, 10% triethylamine in hexane:ether, 1:1 for the trans and 20% of triethylamine for the cis isomer) to yield trans-α-OHTOR (215 mg; 30%) and cis-α-OHTOR (107 mg; 20%). NMR results for trans-α-OHTOR (δ, ppm, CDCl3): 1H-NMR: 2.23 [s, 6H, N(CH3)2], 2.61 [t, 2H, J = 5.8 Hz, CH2-N(CH3)2], 3.40 (m, 2H, CH2-Cl), 3.89 (t, 2H, 5.8 Hz, O-CH2), 4.84 (dd, 1H, J = 6.4 and 6.2 Hz, CH-OH), 6.56 (d, 2H, J = 8.8 Hz, H 3,5 of CC6H4(O), 6.82 (d, 2H, J = 8.8 Hz, H 2,6 of CC6H4(O), and 7.14–7.34 (m, 10H, 2Ph); and 13C-NMR: 45.65 [N(CH3)2], 47.35 [CH2-N(CH3)2], 57.98 (CH2-Cl), 65.46 (O-CH2), 71.74 (CH-OH), and (113.32, 126.73, 127.12, 127.78, 128.19, 129.51, 130.88, 131.42, 134.15, 137.02, 127.76, 141.49, 144.03, 157.07), aromatic and olefin carbons.

Synthesis of α-AcetoxyTOR. Acetic anhydrate (1 ml; 1 mmol) and 4-dimethylaminopyridine (10 mg) were added to a solution of the trans-α-OHTOR (110 mg; 0.26 mmol) in dry triethylamine (2.5 ml). The reaction mixture was stirred at room temperature for 12 h, and then concentrated and purified by column chromatography using silica gel (elucent, 10% triethylamine in hexane:ether, 1:1) to yield α-acetoxyTOR (79 mg; 65%). NMR results for trans-α-OHTOR: (δ, ppm, CDCl3): 1H-NMR: 1.92 (s, 3H, COCH3), 2.22 [s, 6H, N(CH3)2], 2.57 [t, 2H, J = 5.8 Hz, CH2-N(CH3)2], 3.45 (m, 2H, CH2-Cl), 3.85 (t, 2H, J = 5.8 Hz, O-CH2), 5.85 (dd, 1H, J = 6.4 and 6.5 Hz, CH-OAc), 6.50 (d, 2H, J = 8.8 Hz, H 3,5 of CC6H4(O), 6.76 (d, 2H, J = 8.8 Hz, H 2,6 of CC6H4(O), and 7.18–7.42 (m, 10H, 2Ph); and 13C-NMR: 20.78 (COCH3), 43.46 [CH2-N(CH3)2], 45.73 [N(CH3)2], 58.09 (CH2-Cl), 65.57 (O-CH2).

Fig. 1. Structures of TAM and TOR.

Fig. 2. Mechanism for the formation of TAM-DNA adducts.
74.23 (CH-OAc), and (113.33, 126.88, 127.34, 127.86, 128.22, 129.23, 130.70, 131.29, 133.40, 137.71, 141.18, 157.29, aromatic and olefin carbons), and 169.90 (C=O).

Preparation of STa. A rat liver STa (99.2 units/mg protein; 39) and a recombinant STa (62 units/mg protein; Ref. 40) were prepared as described previously. Enzyme units are expressed as nanomoles of sulfuric acid ester product formed from dehydroepiandrosterone per min (39, 40).

Sulfonation of α-OHTAM or α-OHTOR by Hydroxysteroid Sulfotransferases. A trans-form of α-OHTAM was synthesized by the established protocol (38). PAPS was purified by chromatography on DEAE-cellulose according to a published procedure (41). Both α-OHTAM and α-OHTOR were evaluated as substrates of recombinant STa using a published HPLC procedure for determination of the concentration of PAP in the reaction (42). The assay mixtures (30 μl, total volume) contained 0.25 M potassium phosphate buffer (pH 7.0) containing 2.4 mM CaCl₂, using 15 units of micrococcal nuclease and 0.1 units of spleen phosphodiesterase, and incubated for an additional 1 h with 1 unit of nuclease P1, as described previously (20). After the reaction, 100 μl of distilled water was added to the digested sample and extracted twice with 100 μl of butanol. The butanol fractions were back-extracted with 50 μl of distilled water. The butanol fractions were evaporated to dryness and used for analysis of DNA adducts.

Reactivity of α-OHTAM or α-OHTOR with DNA. α-Acetoxy-TAM was synthesized by the established protocol (22). Calf thymus DNA (10 μg) was reacted at 37°C for 1 h with variable amounts of α-acetoxyTAM or α-acetoxyTAM in 100 μl of 0.25 M potassium phosphate buffer (pH 7.0) containing 8.3 mM mercaptoethanol. After the reaction, the DNA was recovered by phenol/chloroform extraction and used for determination of DNA adducts using 32P-postlabeling/HPLC analysis.

Enzymatic Digestion of DNA Samples. DNA sample (0.1–3.0 μg) was enzymatically digested at 37°C for 2 h in 30 μl of 17 mM sodium succinate buffer (pH 6.0) containing 8 mM CaCl₂, using 15 units of micrococcal nuclease and 0.1 units of spleen phosphodiesterase, and incubated for an additional 1 h with 1 unit of nuclease P1, as described previously (20). After the reaction, 100 μl of distilled water was added to the digested sample and extracted twice with 100 μl of butanol. The butanol fractions were back-extracted with 50 μl of distilled water. The butanol fractions were evaporated to dryness and used for analysis of DNA adducts.

Table 1  Formation of DNA adducts via STa-catalyzed sulfonation of α-OHTAM or α-OHTOR

<table>
<thead>
<tr>
<th>α-OHTAM</th>
<th>dG-N²-TAM (adducts/10⁶ nucleotides)</th>
<th>cis-form</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trans-form</td>
<td>fr-1</td>
<td>fr-2</td>
</tr>
<tr>
<td>- STa</td>
<td>n.d.</td>
<td>0.19 ± 0.02</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>+ STa</td>
<td>3.0 ± 0.2</td>
<td>16 ± 1.3</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>α-OHTOR</th>
<th>dG-TOR (adducts/10⁶ nucleotides)</th>
<th>cis-form</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trans-form</td>
<td>fr-a</td>
<td>fr-b</td>
</tr>
<tr>
<td>+ STa</td>
<td>n.d.</td>
<td>0.10 ± 0.02</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

N.D., not detectable.
Detection of DNA Adducts by $^{32}$P-postlabeling/HPLC Analysis. The pooled extracts of digests were incubated at 37°C for 40 min with 30 μCi of [γ-$^{32}$P]ATP and 3 μl of T4 polynucleotide kinase (10 units/μl; Ref. 43) and subsequently incubated for 1 h with potato apyrase (8 units/ml) and eluted over 40 min at a flow rate of 1.0 ml/min with an isocratic solution of 2.0 M ammonium formate (pH 4.0), containing 20% acetonitrile:methanol (6:1; v/v), after which a linear gradient of 20 – 45% was applied to the column for 25 min. Radioactivity was monitored using a Berthold LB506 C-1 radioisotope detector (ICON Scientific, Inc.) connected to a Waters 990 HPLC instrument. Standards of dG3$^3$-TAM (22) were prepared using published methods and labeled with $^{32}$P (43, 44). The relative adduct levels were calculated according to Levay et al. (44), using dpm instead of cpm: (total dpm in adducts)/1.36 × 10$^{11}$ dpm, assuming that 3 $^{32}$P of DNA was 9.09 × 10$^{11}$ pmol of dN$^3$-TAM. The specific activity of the [γ-$^{32}$P]ATP was 1.50 × 10$^7$ dpm/pmol. The specific activity of the [γ-$^{32}$P]ATP was corrected by calculating the extent of decay. A peak counting >200 dpm by radioisotope detector was judged to be significant. The limit of detection of DNA adducts using this technique was 1.5 adducts/10$^9$ nucleotides.

**RESULTS**

**Formation of DNA Adducts via Sulfonation of α-OHTAM or α-OHTOR.** To explore the formation of DNA adducts through $\beta$-sulfonation of α-OHTAM or α-OHTOR, these compounds were incubated with calf thymus DNA, PAPS, and with or without rat STa. When STa was incubated with α-OHTAM, three TAM-DNA adducts were detected at 21.7 min (fr-1), 25.7 min (fr-2), and 59.6 min (fr 3 & 4), respectively (Fig. 3A). The $t_{R}$ of fr-1 and fr-2 were consistent with those of the two diastereoisomers of trans-dG-N$^2$-TAM and the $t_R$ of fr 3 & 4 was consistent with that of a mixture of two diastereoisomers of cis-dG-N$^2$-TAM (Fig. 3C). The level of fr-2 (16 adducts/10$^6$ nucleotides) was higher than the other adducts (Table 1). The total amounts of dG-N$^2$-TAM adducts were 21 adducts/10$^6$ nucleotides. With α-OHTAM alone, a small amount (total, 0.27 adducts/10$^6$ nucleotides) of TAM adducts were detected. No DNA adducts were detected when DNA was incubated without α-OHTAM and STa (data not shown).

In contrast, when α-OHTOR was incubated with STa, only a trace of DNA adduct (0.1 adduct/10$^9$ nucleotides) was detected at 11.0 min (Table 1 and Fig. 3B). The $t_R$ was consistent with that of fr-b, observed when dG$_{1P}$ was reacted with large amounts of α-acetoxy-TOR (Fig. 3D). Without STa, no DNA adduct was detected (Table 1). The level of DNA adducts formed by sulfonation of α-OHTOR was 210 times lower than that for α-OHTAM.

Using hHST, α-OHTAM or α-OHTOR was also incubated under reaction conditions containing DNA and PAPS. With α-OHTAM, the formation of dG-N$^2$-TAM adducts was increased, depending on the amounts of hHST used (Table 2). However, with α-OHTOR, a trace of DNA adduct can be detected only when 0.08 unit of hHST was used (Table 2). The level of TOR adduct was 30 times lower than that of TAM adducts. Like STa, hHST catalyzes α-OHTAM, but not α-OHTOR.

<table>
<thead>
<tr>
<th>α-acetoxyTAM</th>
<th>fr-1</th>
<th>fr-2</th>
<th>fr-3 and 4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 pmol</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
</tr>
<tr>
<td>2.5</td>
<td>1.3 ± 0.05</td>
<td>11 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>15.0</td>
</tr>
<tr>
<td>25</td>
<td>2.8 ± 0.2</td>
<td>62 ± 1.5</td>
<td>6.6 ± 0.4</td>
<td>71.0</td>
</tr>
<tr>
<td>250</td>
<td>6.9 ± 0.3</td>
<td>78 ± 3.2</td>
<td>14 ± 0.8</td>
<td>99.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>α-acetoxyTOR</th>
<th>fr-a</th>
<th>fr-b</th>
<th>fr-c</th>
<th>fr-d</th>
<th>fr-e</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 pmol</td>
<td>0.021 ± 0.005</td>
<td>0.021 ± 0.004</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>0.042</td>
</tr>
<tr>
<td>25</td>
<td>0.042 ± 0.003</td>
<td>0.063 ± 0.005</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>0.11</td>
</tr>
<tr>
<td>250</td>
<td>0.23 ± 0.01</td>
<td>0.57 ± 0.03</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>n.d.$^a$</td>
<td>0.80</td>
</tr>
</tbody>
</table>

$^a$ N.D., not detectable.
Sulfonation of α-OHTAM or α-OHTOR Catalyzed by STa. α-OHTAM or α-OHTOR was incubated with a recombinant STa in the presence of PAPS to monitor the substrate-dependent formation of PAP in the reaction. When α-OHTAM was used, the increased formation of PAP from PAPS was observed, as compared with the control. The rate of α-OHTAM-dependent PAP formation was 15 ± 6 nmol/min/mg STa. In contrast, with α-OHTOR, no significant PAP formation was detected. PAP was not formed even with 5-fold higher amounts of α-OHTOR (500 μM). Apparently unlike α-OHTAM, α-OHTOR cannot serve as a substrate for STa.

Reactivity of α-AcetoxyTOR with dN3p.*dG3p. (0.5 mg) was incubated at 37°C for 4 h with α-acetoxyTOR (0.5 mg) in 200 μl of 100 mM Tris-HCl buffer (pH 7.5), resulting in the formation of five adducts (a: tR = 4.3 min; b: tR = 11.0 min; c: tR = 24.9 min; d: tR = 27.8 min; and e: tR = 59.8 min; Fig. 3D). The level of adducts a, b, c, d, and e were 2.0, 9.0, 0.88, 11.6, and 0.46 adducts/108 nucleotides, respectively. No adducts were observed with other deoxynucleoside 3′-monophosphates (data not shown).

Reactivity of α-AcetoxyTAM or α-AcetoxyTOR with DNA. To compare the reactivity of α-acetoxyTAM to DNA with that of α-acetoxyTOR, calf thymus DNA was reacted with variable amounts of α-acetoxyTAM or α-acetoxyTOR, and the formation of DNA adducts was determined by 32P-postlabeling/HPLC analysis (Table 3). When 250 pmol of α-acetoxyTAM was used, the trans-diastereoisomers (fr-1 and fr-2) of dG-N2-TAM and the cis-form (a mixture of fr-3 and fr-4) were formed (Fig. 4B); the total amounts of TAM adducts were 99 adducts/106 nucleotides (Table 3). Using the equivalent amount of α-acetoxyTOR, a small amount of two adducts were detected (Fig. 4C and Table 3). The tR s of the two adducts were consistent with that of fr-a and fr-b observed when dG3p was reacted with α-acetoxyTOR. The total amount of TOR adducts (0.8 adducts/106 nucleotides) was 125 times lower than those induced by α-acetoxyTAM (Table 3). No adducts were observed with control DNA (Fig. 4A). Because the reactivity of α-acetoxyTOR with dG3p was very poor, the structures of α-acetoxyTOR-modified dG3p could not be identified by LC/MS/MS even when excess amounts of α-acetoxyTOR were reacted.

DISCUSSION

TAM-DNA adducts are primarily formed from α-hydroxylated TAM metabolites including α-OHTAM via sulfonation catalyzed by hydroxysteroid sulfotransferases (20, 21). Because α-hydroxylated TOR metabolites such as α-OHTOR and α-hydroxy-N-desmethyl-TOR were detected in rat urine (35), α-hydroxylated TOR metabolites may be precursors forming TOR-DNA adducts. However, unlike α-OHTAM, α-OHTOR produced only a small amount of DNA adducts under reaction conditions containing PAPS, DNA, and either STa or hHST. This result may be based on the fact that α-OHTOR, a poor substrate for hydroxysteroid sulfotransferases, is not readily converted to the sulfuric acid ester when compared with α-OHTAM.

Fig. 5 Formation of TAM or TOR carbocation intermediates.

Fig. 4. Formation of DNA adducts by reacting DNA with either α-acetoxyTAM or α-acetoxyTOR. Calf thymus DNA (10 μg) was reacted at 37°C for 1 h with either 0.1 μg (250 pmol) of α-acetoxyTAM (B) or α-acetoxyTOR (C) in 200 μl of 100 mM Tris-HCl buffer (pH 7.5). A control incubation (A) with all components except for the α-acetoxy derivatives was also carried out. The DNA was recovered by phenol/chloroform extraction and then analyzed by 32P-postlabeling/HPLC method, as described in “Materials and Methods.”

Fig. 5. Formation of TAM or TOR carbocation intermediates.
This may be one of reasons why TOR is less genotoxic for rats than TAM (11, 36, 45).

Because the reactivity of α-acetoxyTAM with either dG or DNA was similar to that of TAM α-sulfate (22), α-acetoxyTAM can be used as a model activated form of TOR. However, the level of DNA adducts formed by α-acetoxyTOR was much lower than that of α-acetoxyTAM. Our results were consistent with the facts that TOR produced two-orders of magnitude lower levels of DNA adducts in rat liver compared with TAM (11, 36, 45) and no DNA adducts in the mouse liver (46). The lower genotoxicity of TOR may be attributable to the lower reactivity of α-acetoxyTOR with DNA may be attributable to the steric hindrance caused by the presence of a bulky chlorine atom positioned at the ethyl moiety of TOR. In addition, the lower genotoxicity of TOR may be attributable to the lower reactivity of α-acetoxyTAM because of the absence of the ethyl moiety, and it may minimize the formation of the ethyl J biomarker that reacts with the N° position of dG residues in DNA (48).

Both TAM and TOR have similar estrogenic activity in endometrial cancer cells (49) and in rat uterus (50) and produce similar increase in the endometrial thickness of postmenopausal breast cancer patients (51). However, thus far there is no clinical report that TOR increases the incidence of endometrial cancer, although TOR has been used for breast cancer therapy since 1988 in Finland, since 1994 in Sweden, and since 1995 in Japan. This may indicate that DNA damage induced by TAM is primarily involved in the development of endometrial cancer. Because clinical efficacy of TOR for breast cancer patients are similar to that of TAM (52), the use of TOR, instead of TAM, may minimize incidences of endometrial cancer.

RAL, a selective estrogen response modifier, was approved in 1998 by the Food and Drug Administration to use for osteoporosis, but thus far has not been approved for breast cancer therapy. RAL is unlikely to react with DNA because of the absence of the ethyl moiety, and it does not produce proliferative effects on the uterus of postmenopausal women (53). On the basis of a recent RAL chemoprevention trial, RAL also reduced the incidence of breast cancer in women at high risk of developing of breast cancer (54). Unlike TAM, no increased incidence of endometrial cancer was observed in this trial (54). RAL may be another alternative to diminish the risk of development of endometrial cancer.

Because TAM-DNA adducts detected in the endometrium of women treated with TAM (29, 30) have a strong miscoding and high risk of developing breast cancer (54). However, thus far there is no clinical report that TOR increases the incidence of endometrial cancer, although TOR has been used for breast cancer therapy since 1988 in Finland, since 1994 in Sweden, and since 1995 in Japan. This may indicate that DNA damage induced by TAM is primarily involved in the development of endometrial cancer. Because clinical efficacy of TOR for breast cancer patients are similar to that of TAM (52), the use of TOR, instead of TAM, may minimize incidences of endometrial cancer.


In addition, the lower genotoxicity of TOR may be attributable to the lower reactivity of α-acetoxyTAM because of the absence of the ethyl moiety, and it may minimize the formation of the ethyl J biomarker that reacts with the N° position of dG residues in DNA (48).

Both TAM and TOR have similar estrogenic activity in endometrial cancer cells (49) and in rat uterus (50) and produce similar increase in the endometrial thickness of postmenopausal breast cancer patients (51). However, thus far there is no clinical report that TOR increases the incidence of endometrial cancer, although TOR has been used for breast cancer therapy since 1988 in Finland, since 1994 in Sweden, and since 1995 in Japan. This may indicate that DNA damage induced by TAM is primarily involved in the development of endometrial cancer. Because clinical efficacy of TOR for breast cancer patients are similar to that of TAM (52), the use of TOR, instead of TAM, may minimize incidences of endometrial cancer.


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Mechanism of Lower Genotoxicity of Toremifene Compared with Tamoxifen

Shinya Shibutani, Anisetti Ravindernath, Isamu Terashima, et al.


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