Tamoxifen reduces endogenous and UV light-induced oxidative damage to DNA, lipid and protein in vitro and in vivo

Huachen Wei1,2, Qiuyin Cai2, Liqun Tian2 and Mark Lebwohl1

1Department of Dermatology, The Mount Sinai Medical Center, 1425 Madison Avenue, Rm 2-23, Box 1047, New York, NY 10029, USA
2Present address: Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL 35294, USA
3To whom correspondence should be addressed
Email: hw@doc.mssm.edu

We have investigated the effect of tamoxifen (TAM) on endogenous or ultraviolet radiation (UVR)-induced oxidative damage to macromolecules in vitro and in vivo. In a system containing calf thymus DNA exposed to a germicidal UV lamp, both TAM and 4-hydroxytamoxifen (4-OH-TAM) inhibited UVR-induced the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in DNA in a dose-dependent manner. At low concentrations, 4-OH-TAM quenched 8-OHdG more potently than TAM. However, the reduction of 8-OHdG by TAM and 4-OH-TAM became similar at a concentration of 10 µM. In contrast, ascorbic acid had the similar effect to TAM, whereas glutathione exhibited little effect on UVR-induced 8-OHdG. The order of quenching efficacy was: 4-OH-TAM > TAM > ascorbic acid > glutathione. We have further determined the effect of TAM on endogenous 8-OHdG formation, lipid peroxidation, and protein oxidation in the skin of SENCAR mice. Topical application of 5 µmol TAM significantly reduced the level of 8-OHdG in mouse epidermis by ~27% (P < 0.05). Endogenous lipid peroxidation and protein oxidation, measured as malondialdehyde (MDA) and carbonyl groups, were also substantially reduced by topical TAM. Further study was conducted to evaluate the effect of TAM on UVR-induced 8-OHdG and MDA in skin of hairless mice. In mice subacutely exposed to low dose (3.4 kJ/m² UVR-induced 8-OHdG and MDA in skin of hairless mice, the study was conducted to evaluate the effect of TAM on were also substantially reduced by topical TAM. Further endogenous lipid peroxidation and protein oxidation, measured as malondialdehyde (MDA) and carbonyl groups, were also substantially reduced by topical TAM.

Abbreviations: TAM, tamoxifen; 4-OH-TAM, 4-hydroxytamoxifen; TPA, 12-O-tetradecanoyl phorbol-13-acetate; UVR, ultraviolet radiation; ROS, reactive oxygen species; H2O2, hydrogen peroxide; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; dG, deoxyguanosine; DMSO, dimethyl sulfoxide; DNPH, 2,4-dinitrophenylhydrazine; PMNs, polymorphonuclear leukocytes; ECD, electrochemical detection.

Introduction

Tamoxifen (TAM*), an antiestrogen, is widely used as an adjuvant in the treatment of breast cancer (1,2). TAM has undergone clinical trials in Europe, as well as in the United States and Canada, to evaluate its preventive effect on breast cancer in women at high risk (3–5). Recently, TAM has been used to treat other cancers, such as liver (6), brain (7) and pancreas (8). It has been reported that TAM and its active metabolite 4-hydroxytamoxifen (4-OH-TAM, structure see Figure 1) exert anti-oxidative effects in vitro. TAM and 4-OH-TAM inhibit metal ion-mediated lipid peroxidation in rat liver microsomes and in phospholipid liposomes (9,10). Furthermore, Wiseman and Halliwell (11) reported that TAM and 4-OH-TAM protected rat liver nuclei against Fe(III)-ascorbate dependent lipid peroxidation. Cust’dio et al. (12) found that TAM and 4-OH-TAM were efficient inhibitors of lipid peroxidation induced by Fe(II)/ascorbate, and the strong intramembranous scavengers of peroxyl radicals generated by the watersoluble 2,2'-azobis(2-amidinopropane)dihydrolucitol and by lipid-soluble 2,2'-azobis(2,4-dimethylvaleronitrile). Recently, Thangaraju et al. (13) observed that TAM decreased lipid peroxidation in post-menopausal women with breast cancer. TAM also has been reported to suppress tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced hydrogen peroxide (H2O2) formation by human neutrophils (14) and HeLa cells (15), and H2O2 formation in the skin of SENCAR mice (16).

Many biological events such as metabolism of xenobiotics, phagocytosis, excessive exercises, exposure to redox chemicals, and ionizing and ultraviolet radiation (UVR) can trigger the generation of reactive oxygen species (ROS) in vivo and in vitro. These ROS, if excessively produced and not timely scavenged, will cause extensive oxidative damage to biological macromolecules, including DNA, RNA, protein and lipid. Oxidative DNA damage bears more biological relevance since it is implicated in carcinogenic and aging processes (17–19). One of the most frequently monitored markers of oxidative DNA damage is 8-hydroxy-2'-deoxyguanosine (8-OHdG). The formation of 8-OHdG can be induced by various carcinogens, ionizing irradiation and UV light radiation (19–24).

However, whether TAM and its metabolites modulate endogenous oxidative stress and UVR-induced oxidative damage to macromolecules remains unknown. Thus, the objective of the present study is to determine if TAM and its active metabolite 4-OH-TAM affect UVR-induced 8-OHdG formation in vitro, and endogenous and UVR-induced oxidative modification of DNA, lipid, and protein in vivo.

Materials and methods

Chemicals and reagents

TAM was purchased from Aldrich Chemical Co. (Milwaukee, WI). 4-OH-TAM was purchased from Research Biochemicals International (Natick, MA). Ascorbic acid, glutathione, calf thymus DNA, deoxyguanosine (dG), dimethyl sulfoxide (DMSO), 2,4-dinitrophenylhydrazine (DNPH), thiobarbituric acid (TBA) and malondialdehyde (MDA) were purchased from Sigma Co. (St Louis, MO). Nuclease P1 and alkaline phosphatase were purchased from Boehringer Mannheim Co. (Indianapolis, IN).

UV irradiation of calf thymus DNA

Calf thymus DNA was solubilized in 10 mM Tris-HCl (pH 7.0) to a concentration of ~200 µg/ml. The solutions (total volume of 5 ml) containing
400 µg DNA and different concentrations of quenchers (TAM, 4-OH-TAM, ascorbic acid and glutathione) were added in 6-well plastic cell culture flask (3.4 cm diameter) and exposed to a 15T8 germicidal lamp (General Electronics) 20 cm away from the solution surface for 10 min. The UV light contains an 85% of UVC (254 nm) and an 15% of UVA (365 nm). The radiation energy was 7 kJ/m² as measured by a Model IL 1700 research radiometer from International Light, Inc. (Newburyport, MA). After irradiation, 5 M NaCl was added to the samples to reach a final concentration of 1 M, followed by adding two volumes of ice-cold ethanol to the samples. DNA was then precipitated by centrifugation at 12 000 g for 10 min. The DNA pellet was washed with 70% ethanol, dried in the air and then solubilized in 0.5 ml of 10 mM Tris-HCl (pH 7.0) for analyses of 8-OHdG.

Maintenance and treatment of animals

Female SENCAR mice at age 6 weeks were purchased from the Biological Testing, National Cancer Institute (Frederick, MD) and accommodated at the animal facility for 1 week for environmental adjustment. Mice were kept under standard conditions (12 h light/12 h dark cycle, humidity at 50 ± 15%, temperature 22 ± 2°C and air changes/h). The dorsal hair of the mice was shaved with surgical clippers 48 h prior to experiments. Different doses of TAM in 0.2 ml vehicle (acetone) or vehicle alone were topically applied to shaved with surgical clippers 48 h prior to experiments. Different doses of TAM or vehicle alone prior to exposure to low-dose (3.4 kJ/m², µmol of TAM or vehicle alone prior to exposure to low-dose (3.4 kJ/m², or 3.5, 1.5 µmol of 8-OHdG). To make data comparable, the results are normalized to the percentage over the positive control. Briefly, 0.1 ml of homogenates was added to the test tube containing 0.2 ml of 8.1% SDS, 1.5 µmol of 20% acetic acid, pH 3.5, 1.5 µmol of 0.8% BSA solution and 0.7 ml distilled water. The mixture was heated at 95°C for 1 h. After cooling in ice-water, the samples were extracted with 4 ml of the mixture of n-butanol and pyridine (15:1, v/v), followed by centrifugation at 3000 rpm for 10 min. The organic phase was collected and the absorbance measured at a wavelength of 352 nm. The results were calculated based on an MDA standard curve and expressed as nmol MDA equivalent/mg protein.

Determination of lipid peroxidation

Carbonyl groups were assayed as a marker of protein oxidation. The skin homogenates were centrifuged at 12 000 g for 10 min. Supernatant was recovered and the protein concentration was determined using the Pierce BCA protein assay kit. The protein carbonyl groups were analysed by DNS method as described by Levine et al. (28). Briefly, 1 ml of tissue supernatant was pipetted into the tubes, to which 4.0 ml DNPH in 2.5 M HCl was added. The blank was made by adding 2.5 M HCl only. After incubation at room temperature for 1 h, the proteins were precipitated by adding 5 ml of 5% trichloro-acetic acid and washed with 4 ml of ethanol:ethyl acetate (1:1) three times. Precipitated protein was redisolved in 2.0 ml of 6 M guanidine HCl, 20 mM potassium phosphate, pH 6.5. Insoluble materials were removed by centrifugation at 3000 rpm. Carboxyl groups were calculated from the maximum absorbance (360–370 nm) using a molar absorption coefficient of 22 000 M/cm, expressed as nmol of carbonyl groups/mg protein.

Results

Effect of TAM and 4-OH-TAM on 8-OHdG formation-induced by UV irradiation

We have previously shown that a germicidal UV lamp substantially increased the formation of 8-OHdG in purified DNA (22–24). In the present study, irradiation of calf thymus DNA at a dose of 7 kJ/m² significantly increased the level of 8-OHdG by >150-fold as compared to the untreated DNA (8-OHdG/10⁵ dG of 552.3 versus 3.6). To make data comparable, the results are normalized to the percentage over the positive controls (without the scavengers). As shown in Figure 2, both TAM and 4-OH-TAM quenched UV light-induced 8-OHdG in a dose-dependent manner. At the concentrations below 10 µM, the quenching effect of 4-OH-TAM was much more pronounced than that of TAM. The IC₅₀ of quenching UVR-induced 8-OHdG formation was 0.6 µM for 4-OH-TAM, as compared to 2.5 µM for TAM. With the concentration increasing up to 10 µM, the difference in quenching efficacy between TAM and 4-OH-TAM diminished. Two classic anti-oxidants, ascorbic acid and glutathione were also tested in this system. Ascorbic acid exhibited a similar dose-response pattern to TAM, whereas glutathione had little effect on UVR-induced 8-OHdG formation. These results indicate that TAM and its active metabolite 4-OH-TAM have similar or better protective effects on UVR-induced 8-OHdG in comparison to ascorbic acid and glutathione.
Inhibition of oxidative damage by tamoxifen

Effect of TAM on endogenous formation of 8-OHdG in the mouse skin

SENCAR mice have relatively high endogenous oxidative status compared to other strains of mice as evidenced by production of high levels of H$_2$O$_2$ by phagocytes (29) and high basal levels of H$_2$O$_2$ and 8-OHdG in mouse epidermis (30). Thus, we examined the effect of TAM on the levels of endogenous 8-OHdG in the skin of SENCAR mouse. Topical application of TAM moderately reduced the levels of 8-OHdG in the mouse skin 6 h post treatment with a maximum inhibition of 27% ($P < 0.05$) at 10 µmol TAM (Table I). Table II shows the time courses of 8-OHdG level in mouse skin treated with 5 µmol TAM. The levels of 8-OHdG were significantly decreased at 6 h and the significant reduction of endogenous 8-OHdG remained up to 24 h post-TAM treatment.

Effect of TAM on endogenous oxidation of lipids and proteins in mouse skin

Topical application of TAM significantly reduced the levels of MDA (a marker of lipid peroxidation) and carbonyl groups (a marker of protein oxidation) in SENCAR mouse skin. As shown in Figure 3, endogenous lipid peroxidation was significantly reduced by 66% ($P < 0.05$) at 10 µmol TAM and 82% ($P < 0.01$) at 100 µmol TAM, respectively. In addition, endogenous protein oxidation in mouse skin, measured as the carbonyl groups were decreased by 27% ($P < 0.05$) at 10 µmol TAM and by 55% ($P < 0.01$) at 100 µmol TAM, respectively (Figure 4). It appears that reduction of endogenous lipid peroxidation by TAM is more pronounced than that of protein oxidation.

Effect of TAM on UVR-induced 8-OHdG and MDA in hairless mouse

Acute and subacute exposure to UVR irradiation were known to increase the level of 8-OHdG in hairless mouse skin (25,26). In this study, mice were topically treated with 10 µmol of TAM prior to exposure to low-dose (3.4 kJ/m$^2$, three times a week for six doses) and high-dose (16.8 kJ/m$^2$, three times a week for three doses) of UVR irradiation. As shown in Table III, topical TAM significantly reduced the levels of UVR-induced 8-OHdG by 75 and 81%, respectively, in epidermis exposed to low and high UVR. In addition, TAM exhibited a similar inhibition of UVR-induced MDA in skin of hairless mice by 37 and 65%, respectively (Table IV).

---

**Table I. Effect of TAM on endogenous 8-OHdG formation in skin of SENCAR mice**

<table>
<thead>
<tr>
<th>TAM (µmol)</th>
<th>n</th>
<th>8-OHdG/105 dG Mean ± SD</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>9.38 ± 0.94</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>8.51 ± 0.26</td>
<td>9.3</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>8.62 ± 0.62</td>
<td>8.1</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>8.59 ± 0.36</td>
<td>8.5</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>6.89 ± 0.62</td>
<td>26.5</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>8.04 ± 0.23</td>
<td>14.3</td>
</tr>
</tbody>
</table>

*a*Mouse skin was treated with 5 µmol TAM in 0.2 ml of acetone as described in Materials and methods. 
bStatistically significant versus the control group by two-tailed Student’s t-test ($P < 0.05$).

**Table II. Time course of TAM on endogenous 8-OHdG formation in skin of SENCAR mice**

<table>
<thead>
<tr>
<th>Time post-TAM treatment (h)</th>
<th>n</th>
<th>8-OHdG/10$^5$ dG Mean ± SD</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>9.42 ± 0.40</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>8.46 ± 0.22</td>
<td>10.2</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>8.16 ± 0.29</td>
<td>13.4</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>7.37 ± 0.43</td>
<td>21.8</td>
</tr>
<tr>
<td>24</td>
<td>4</td>
<td>7.54 ± 0.36</td>
<td>19.7</td>
</tr>
</tbody>
</table>

**Table III. Effect of TAM on endogenous lipid peroxidation in the mouse skin**

<table>
<thead>
<tr>
<th>TAM (µmol)</th>
<th>n</th>
<th>MDA (µmol/mg protein) Mean ± SD</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>0.17 ± 0.06</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>0.36 ± 0.02</td>
<td>26.5</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0.62 ± 0.03</td>
<td>48.4</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>0.62 ± 0.03</td>
<td>48.4</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.26 ± 0.02</td>
<td>66.7</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>0.27 ± 0.02</td>
<td>78.9</td>
</tr>
</tbody>
</table>

*a*Statistically significant versus the control group by two-tailed Student’s t-test ($P < 0.05$). 
bStatistically significant versus the control group by two-tailed Student’s t-test ($P < 0.01$).

**Table IV. Effect of TAM on endogenous protein oxidation in the mouse skin**

<table>
<thead>
<tr>
<th>TAM (µmol)</th>
<th>n</th>
<th>Carbonyl groups Mean ± SD</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>0.62 ± 0.03</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>0.36 ± 0.02</td>
<td>26.5</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0.62 ± 0.03</td>
<td>48.4</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>0.62 ± 0.03</td>
<td>48.4</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.26 ± 0.02</td>
<td>66.7</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>0.27 ± 0.02</td>
<td>78.9</td>
</tr>
</tbody>
</table>

*a*Statistically significant versus the control group by two-tailed Student’s t-test ($P < 0.05$). 
bStatistically significant versus the control group by two-tailed Student’s t-test ($P < 0.01$).
Fig. 4. Effect of TAM on endogenous protein oxidation in the mouse skin. SENCAR mice (four per group) were topically treated with TAM and killed 12 h post-treatment. Skin was removed and carbonyl contents were determined 12 h post-treatment and protein oxidation was determined as described in Materials and methods. The background level of carbonyl contents in untreated mouse skin is 7.51 ± 1.37 (mean ± SD, n = 4).

Discussion

Tamoxifen has drawn wide attention in recent years because of its therapeutic and chemopreventive effect on breast cancer (1–5). Recently, TAM has been used to treat other non-breast cancers (6–8), suggesting that TAM might have general mechanisms in the prevention and treatment of cancers. A number of studies have shown that TAM and 4-OH-TAM inhibit lipid peroxidation in vitro (9–12) and suppress carcinogen-induced H₂O₂ production in vivo and in vitro (14–16). We hypothesize that TAM may exert its anti-cancer and chemopreventive effects through protection of macromolecules against damage by endogenous and exogenous oxidants. In the present study, experiments have been designed to investigate the effect of TAM on endogenous and UV-induced oxidative DNA damage, lipid peroxidation and protein oxidation in vitro and in vivo.

Endogenous or chemical-mediated production of ROS may differ from that generated by UV irradiation in that the former usually generates hydroxyl radicals as an ultimate reactive species, and the latter predominantly produces singlet oxygen targeting DNA molecules (31,32). In the present study, TAM and its active metabolite 4-OH-TAM substantially blocked UV light-induced 8-OHdG formation in vitro more potently than classic anti-oxidants ascorbic acid and glutathione. Overall potency order of the tested compounds was: 4-OH-TAM > TAM ≈ ascorbic acid > glutathione. It appears that UV-induced oxidation of dG base in DNA is not through generation of hydroxyl radicals. The potent quenching effect on UVR-induced 8-OHdG by TAM and 4-OH-TAM cannot be explained by their anti-oxidant activities since both compounds have very weak scavenging capacity on H₂O₂ (data not shown). We assume that TAM and 4-OH-TAM quench UVR-induced 8-OHdG formation by scavenging singlet oxygen generated by UV radiation, although this hypothesis needs to be confirmed in future studies. In addition, 4-OH-TAM displays a more potent quenching of 8-OHdG than its parent TAM, which correlates well with their inhibition of lipid peroxidation in isolated nuclei (9), suggesting that metabolism of TAM via hydroxylation of 8-OHdG formation at C4 position may enhance the protection of UVR-mediated oxidative damage.

A concern may be raised as to the sunscreening effect of TAM rather than a quenching effect on UV-induced 8-OHdG. It is possible that TAM absorbs UV light better than DNA. To test if TAM is a potent UV absorber or not, we conducted a UV absorbance test. The results showed that TAM did not block UVB penetration at concentrations up to 100 µM. Therefore, the concern that TAM blocks UV light should be minimal. In addition, DNA is an excellent UV absorber in that its absorption spectrum overlaps with the wavelengths of UVB and C. In the present study, 10 µM TAM quenched >95% of UV-induced 8-OHdG at a TAM:DNA molar ratio of 6.25×10⁻³ (1 TAM versus 160 DNA molecules). Therefore, at such a low concentration of TAM and 4-OH-TAM, the potent reduction of UV-induced 8-OHdG is less likely attributed to the direct blocking or absorbing effect of TAM.

UVR-induced oxidative DNA damage in vivo may represent a combined mechanism involving direct and indirect actions. Direct UV action is known to generate singlet oxygen and...
possibly other ROS (31,32), while indirect UV action is manifested as the inflammatory responses post-UV radiation, including erythema and infiltration of polymorphonuclear leukocytes (PMNs). The infiltrated PMNs are able to generate superoxide anion and \( \text{H}_2\text{O}_2 \) and UV irradiation is known to stimulate the release of cytokines in keratinocytes. TAM has been shown to inhibit TPA-induced production of \( \text{H}_2\text{O}_2 \) in HL-60 and HeLa cells (14,15) and reduce TPA-induced cutaneous inflammation (16). Therefore, TAM may have dual effects on the formation of 8-OHdG in UV-irradiated skin by scavenging singlet oxygen and inhibiting ROS release by inflammatory cells.

The inhibition of TAM on endogenous 8-OHdG formation in the skin of SENCAR mice gives rise to another mechanism of action. Oxidative modification of DNA bases can be produced by endogenous metabolism. Genetic orientation for overproduction of ROS or deficiency in elimination of ROS would create a high level of pro-oxidative states. SENCAR mice have been shown to have relatively high endogenous oxidative status in that their macrophages produce more ROS (29) and skin tissue has high basal levels of \( \text{H}_2\text{O}_2 \) and 8-OHdG than other mouse strains (30). In the present study, topical application of TAM reduced the levels of endogenous 8-OHdG in mouse skin. This reduction may be due to the inhibition of cellular production of ROS since several in vitro studies showed that TAM suppressed tumor promoter TPA-mediated \( \text{H}_2\text{O}_2 \) production by human PMNs and the cultured HeLa cells (14,15). The current results indicate that TAM can reduce oxidative DNA damage mediated by endogenous oxidative stress.

TAM has been shown to inhibit oxidant-induced lipid peroxidation in vitro (9–12) and reduce lipid peroxidation in post-menopausal women with breast cancer (13). This antioxidant property is proposed to have the link with anti-cancer activity. In the current study, we found that TAM reduced endogenous lipid peroxidation and protein oxidation in the SENCAR mouse skin and UBV-induced lipid peroxidation in hairless mouse skin. Our studies confirmed the other investigators’ studies, in which TAM was shown to inhibit metal-ion induced lipid peroxidation and to protect human low density lipoprotein against oxidative damage (9–12). It appears that TAM has more impact on lipid peroxidation than on protein oxidation in vivo. At a dose of 10 µmol TAM, lipid peroxidation was reduced by 66%, whereas protein oxidation was decreased only by 27%.

Recently, debate over the potential carcinogenicity of tamoxifen has arisen due to the fact tamoxifen was found to increase liver cancer incidence in rats (31,32,33). Several studies (34–37) including one from this laboratory (38) have shown that TAM induces DNA adducts in vivo and in vitro, which raised the concern as to the potential carcinogenicity of TAM in vivo. However, DNA adducts formed by TAM in SENCAR mouse skin display a high polarity as compared to a potent carcinogen 7,12-dimethylbenz[a]anthracene (38). In addition, our pilot animal studies demonstrated that TAM per se or TAM as an initiator plus TPA as a tumor promoter did not induce any skin tumors in SENCAR mice (unpublished data). Thus, TAM-DNA adducts may represent a harmless byproduct of TAM metabolism in skin model. More recently, Ye and Bodell (39) reported that the level of 8-OHdG was increased 3-fold by TAM when DNA was incubated with rat liver microsomal preparations containing reduced NADPH as a co-factor. However, no significant increase of 8-OHdG was observed when cumene hydroperoxide was used as a co-factor. Thus, it should be of great value if the induction of 8-OHdG by TAM is confirmed in an in vivo model. Instead, our study displays a consistent inhibition of UVR-induced 8-OHdG by TAM in vivo and in vitro. Therefore, we conclude that the benefit of TAM may outweigh its detrimental effect in the skin model.

In summary, we have demonstrated that TAM blocks UVR-induced oxidative damage to macromolecules in vivo and in vitro. 4-OH-TAM, the active metabolite of TAM, exhibits more potent quenching of UVR-induced 8-OHdG in vitro than TAM. Oxidative damage to macromolecules such as DNA and lipid is known to be related to carcinogenic processes. Thus, the inhibition of endogenous and UVR-induced oxidative DNA damage and lipid peroxidation in mouse skin may, at least in part, contribute to the anti-carcinogenic and chemopreventive effects of TAM.

Acknowledgements

This research was supported by USPHS grants (R01 CA60994 and R01 CA 61764) and grants from American Institute for Cancer Research (96B001) and Dermatology Foundation awarded to H.Wei.

References


34. Wogan,G.N. Review of the toxicology of tamoxifen. Semin Oncol., 24 (Suppl. 1), S1–S87, S197.


