

# Gender Differences in UVB-Induced Skin Carcinogenesis, Inflammation, and DNA Damage

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## Abstract

**The American Cancer Society reports the incidence of squamous cell carcinoma in males to be thrice the incidence in females. This increased squamous cell carcinoma incidence has been attributed to men accumulating more sun exposure and using less sun protection than women. To date, there have been no controlled studies examining the effect of gender on skin tumor development following equal doses of UVB. Gender differences in UVB-induced skin carcinogenesis were examined using the Skh-1 mouse model. After chronic exposure to equal doses of UVB, male mice developed tumors earlier and had more tumors than female mice; tumors in male mice tended to be larger, and the total tumor burden was greater than in females. In addition, tumors in males were of more advanced histologic grade compared with those of female mice. To evaluate the contribution of differences in inflammation and DNA damage to differences in skin carcinogenesis, male and female Skh-1 mice were exposed once to 2,240 J/m<sup>2</sup> UVB and examined 48 h after exposure. Surprisingly, male mice developed less of an inflammatory response, as determined by skin fold thickness and myeloperoxidase activity, compared with females. Interestingly, male mice showed more cutaneous oxidative DNA damage than the females and lower antioxidant levels. These results show a gender bias in skin carcinogenesis and suggest that the gender difference in tumor development is more influenced by the extent of oxidative DNA damage and antioxidant capacities than by inflammatory response.** [Cancer Res 2007;67(7):3468–74]

## Introduction

Epidemiologic studies have reported the development of significantly more nonmelanoma skin cancers in men than women (1–4), with the American Cancer Society reporting approximately twice the incidence of nonmelanoma skin cancer development in men compared with women.<sup>3</sup> It is currently believed that lifestyle plays a major role in this gender disparity, as males tend to spend more time out in the sun and use less sun protection (5, 6). However, no studies have determined if gender differences in skin tumor development are still observed when males and females receive equivalent cumulative UV radiation exposure. Many studies have shown that chronic exposure to UV radiation leads to the development of nonmelanoma skin cancer (reviewed in ref. 7). Nonmelanoma skin cancer includes basal cell carcinoma and squamous cell carcinoma, which comprise approximately half of all

diagnosed cancers in the United States.<sup>3</sup> The incidence of basal cell carcinoma is 2-fold greater and the incidence of squamous cell carcinoma is 3-fold greater in males than females.<sup>3</sup> Occupational exposure to UV light shows a strong relationship between cumulative sunlight exposure and squamous cell carcinoma risk (8). The UV light B (UVB, 280–320 nm) portion of the solar spectrum contributes 75% of the skin cancer risk, compared with 25% for UV light A (UVA, 320–400 nm) and 0% for UV light C (UVC, 200–280 nm; ref. 9).

Exposure to UVB induces cutaneous inflammation. A single exposure to UVB can result in an acute inflammatory response, which is characterized by erythema (redness) and edema (swelling) due to increased vascular flow and vascular permeability (reviewed in refs. 10, 11). Increases in vascular flow and permeability, in turn, aid in the recruitment and infiltration of inflammatory cells, including neutrophils and monocytes, into the skin (12, 13). Adhesion molecules expressed on vascular endothelial cells are altered to increase adhesion of the inflammatory cells enhancing their infiltration into the skin (13). Neutrophils are the first inflammatory cells recruited to UVB-exposed skin. The activity of myeloperoxidase, an enzyme released by activated neutrophils, is increased during cutaneous inflammation and induces the production of reactive oxygen species (ROS; refs. 14, 15). Although these responses are normal and transient, chronic cutaneous inflammation due to repeated UVB exposures can have negative consequences. Our laboratory and others have previously shown a clear link between UVB-induced inflammation and the development of skin tumors (15–20). Inhibition of this inflammatory response via the topical application of the anti-inflammatory drug, celecoxib, inhibited the acute inflammatory response after a single UVB exposure and decreased tumor formation after chronic exposure (19, 20).

In addition to inducing inflammation in the skin, exposure to UVB results in the formation of both direct and indirect DNA damage. Direct damage includes the formation of cyclobutane pyrimidine dimers (CPD) and (6-4) photoproducts. Exposure to UVB also indirectly induces oxidative DNA damage, in part through the generation of ROS by infiltrating inflammatory cells, including neutrophils and macrophages, and by activated keratinocytes. There is substantial evidence for the role of ROS in skin carcinogenesis (reviewed in ref. 21). The most common type of DNA damage caused by ROS is the formation of 8-oxo-deoxyguanosine (8-oxo-dG) adducts (reviewed in ref. 22); thus, 8-oxo-dG adducts are a well-recognized marker for DNA damage caused by oxidative stress (23). ROS induces oxidative damage when the complex antioxidant mechanisms are overwhelmed (24).

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Failure to adequately repair DNA lesions, both direct and indirect, can result in the formation of skin cancers (25).

Gender differences have been reported in the development of a number of diseases. For example, recent studies suggest that women may be more susceptible to tobacco-induced lung cancer (26). There have also been reported gender differences in the development of chronic myeloid leukemia (27), colon cancer (28), multiple sclerosis (29), and diabetic cardiomyopathy (30). A report of the Surveillance, Epidemiology and End Results program of the National Cancer Institute revealed the cancer incidence for all sites to be greater in males compared with females, and the cancer mortality rate is similarly higher in males (31). Although there have been epidemiologic reports of gender differences in the development of cutaneous squamous cell carcinoma,<sup>3</sup> to date there have been no controlled murine studies examining this difference. The current study used a well-established Skh-1 murine model to compare gender differences in UVB-induced inflammation and skin carcinogenesis in males and females exposed to equal doses of UVB and revealed a significant difference between the sexes in tumor susceptibility.

## Materials and Methods

### Animal Treatments

Male and female outbred Skh-1 mice (Charles River Laboratories, Wilmington, MA) were housed in the vivarium at Ohio State University according to the requirements established by the American Association for Accreditation of Laboratory Animal Care. Procedures were approved by the appropriate Institutional Animal Care Utilization Committee before the initiation of any studies. Irradiated mice were exposed to UVB dorsally with Phillips FS40UVB lamps (American Ultraviolet Company, Lebanon, IN) fitted with Kodacel filters (Eastman Kodak, Rochester, NY) to ensure the emission of UVB light (290–320 nm). The UVB dose was determined by a UVX radiometer (UVB, Inc., Upland, CA). Both male and female mice were housed six animals per cage, and there was no evidence of dorsal wounds caused by fighting.

The chronic UVB study was conducted to examine gender differences in skin carcinogenesis when animals were exposed to equal doses of UVB. Mice ( $n = 6$  per group) were irradiated thrice weekly with 2,240 J/m<sup>2</sup>, previously determined to be 1 minimal erythemic dose (MED) for female Skh-1 mice, for 16 weeks then sacrificed after week 25. Digital calipers were used to measure tumors >1 mm in diameter at weekly intervals; the first measurable tumors appeared in week 6. The time to first tumor was determined to be the time of the first observation of a measurable tumor. Immediately after sacrifice, 0.5 cm<sup>2</sup> skin sections and multiple tumors were harvested and fixed in 10% neutral buffered formalin.

Acute UVB studies were conducted to compare gender differences in the cutaneous inflammatory response and in levels of epidermal DNA damage. Male and female mice were exposed to 2,240 J/m<sup>2</sup> UVB and sacrificed 48 h after exposure. This UVB dose and time point were based on previous studies in our laboratory, demonstrating the peak of the inflammatory response to occur 48 h after exposure in both males and females (data not shown). Additionally, in a dose response study of males and females with a single UVB exposure ranging from 0 to 4,480 J/m<sup>2</sup>, the experimentally determined MED for both males and females was 2,240 J/m<sup>2</sup> (data not shown). Immediately following sacrifice, dorsal skin fold thickness was measured to assess edema, 10-mm punch biopsies were collected to determine myeloperoxidase levels, 0.5 cm<sup>2</sup> skin sections were harvested and fixed in 10% neutral buffered formalin for immunohistochemical analysis, and the remaining dorsal skin was collected and snap frozen in liquid nitrogen for antioxidant analysis.

### Quantitation of Tissue Myeloperoxidase Levels

Myeloperoxidase, an enzyme that converts hydrogen peroxide to hypochlorous acid, is released by activated neutrophils during inflamma-

tory events. The levels of myeloperoxidase in cutaneous tissue were determined biochemically and used as a measure of activated neutrophil infiltration, as previously described (19).

### Immunohistochemical Techniques

Immediately after sacrifice, 0.5 cm<sup>2</sup> skin sections and three tumors from each mouse were placed in 10% neutral buffered formalin (2 h for skin; 4 h for tumors), washed with PBS, processed, and then embedded in paraffin blocks. Tissue sections (5  $\mu$ m) were cut and mounted onto Superfrost Plus microscope slides (Fisher Scientific, Pittsburgh, PA). The tissues were then deparaffinized using Clear-Rite 3 (Richard-Allan Scientific, Kalamazoo, MI) and hydrazined in a graded series of alcohols.

**Immunohistochemical detection of epidermal CPDs.** For CPD detection, rehydrated sections were incubated in 3% H<sub>2</sub>O<sub>2</sub> in 100% methanol for 10 min at room temperature, at 37°C for 10 min in 0.125% trypsin, and in 1 N HCl for 30 min at room temperature, with dH<sub>2</sub>O rinses before and after each step. Sections were then incubated with M.O.M. (Vector Laboratories, Burlingame, CA) reagents, according to kit instructions using an antithymine dimer antibody (1:50, Kamiya Biomedical Co., Seattle, WA), followed by ABC reagent (Vector Laboratories) for 30 min at room temperature. Finally, sections were incubated with diaminobenzadine (DAB) solution (Vector Laboratories) for 30 s, rinsed with dH<sub>2</sub>O, counterstained with hematoxylin 2 (Richard-Allan), dehydrated, and mounted. This protocol was modified from that previously reported (32). CPD-stained sections were analyzed by counting the number of positive and total nuclei in the epidermis from each animal in 10 fields (60 $\times$ ). The data were expressed as the percentage of positive cells per field.

**8-Oxo-dG adduct detection.** For 8-oxo-dG adduct detection, sections were baked at 60°C for 30 min before rehydration. After rehydration, sections were rinsed in dH<sub>2</sub>O, then PBS. Antigen retrieval was done for 20 min in 10 mmol/L sodium citrate buffer (pH 6.0) in a steamer, followed by 20 additional minutes of cooling in the antigen retrieval solution at room temperature. Sections were incubated in RNase solution [1  $\mu$ g/mL RNase in RNase buffer (10 mmol/L Tris, 1 mmol/L EDTA, 0.4 mol/L NaCl, pH 7.5)] with a PBS rinse before and after the incubation. The sections were then blocked in 10% normal rabbit serum (Vector Laboratories) for 1 h at room temperature, incubated with avidin (blocking kit from Vector Laboratories) for 10 min at room temperature, rinsed in PBS, incubated with biotin (blocking kit from Vector Laboratories) for 10 min at room temperature, and finally rinsed again in PBS. The sections were incubated overnight at 4°C with anti-8-oxo-dG antibody (0.75  $\mu$ L/mL in 10% normal rabbit serum, Alpha Diagnostics International, San Antonio, TX). The next day, sections were rinsed with PBS and incubated in biotinylated rabbit anti-goat IgG (0.255  $\mu$ g/mL in 10% normal rabbit serum, Vector Laboratories) for 1 h at room temperature. Sections were rinsed in PBS, incubated in ABC for 30 min at room temperature, and rinsed in PBS. Sections were finally incubated in DAB for 1 min, rinsed in dH<sub>2</sub>O, counterstained with hematoxylin 2, dehydrated, and mounted. Sections stained for 8-oxo-dG were analyzed by counting the number of positive and total nuclei in the epidermis from each animal in 10 fields (60 $\times$ ). The data were expressed as the percentage of positive cells per field.

**Tumor grading.** After sacrifice, three random tumors per irradiated animal were harvested for histologic grading. H&E-stained tissue sections of tumors isolated from UVB-irradiated male and female mice were graded in a blinded manner by a board-certified veterinary pathologist (D.F.K.). Grades were assigned as follows: hyperplasia, papilloma (grades 1–3), microinvasive squamous cell carcinoma (grades 1–3), or fully invasive squamous cell carcinoma. Papillomas were exophytic tumors that showed no evidence of stromal invasion, whereas squamous cell carcinomas had a more endophytic appearance, with stromal invasion evidenced by loss of basement membrane continuity and development of an inflammatory stromal response. A grade 1 papilloma was composed primarily of epithelium without a pronounced papillary pattern; a grade 2 papilloma was a well-differentiated papillary mass; a grade 3 papilloma was similar to a grade 2 papilloma, except that a few finger-like projections of atypical cells at the base of the mass were present. Microinvasive squamous cell carcinomas were subcategorized by depth of penetration into the dermis.

Only tumors that invaded the panniculus carnosus were classified as fully invasive squamous cell carcinomas. Hyperplasia and all grades of papilloma were considered benign, whereas squamous cell carcinoma and all grades of microinvasive squamous cell carcinoma were considered malignant.

### Antioxidant Assay

The antioxidant assay (antioxidant assay, Sigma-Aldrich, St. Louis, MO) was conducted to quantitatively determine the cumulative antioxidant capacity of male and female unirradiated and UVB-exposed skin. Frozen tissue was ground to a powder with a mortar and pestle then  $1 \times$  cold assay buffer was added (20 mg tissue/100  $\mu$ L  $1 \times$  assay buffer). The samples were homogenized, centrifuged at  $12,000 \times g$  for 15 min at  $4^\circ\text{C}$ , and the supernatant was analyzed according to kit instructions. In this assay, metmyoglobin and hydrogen peroxide form a ferryl myoglobin radical, which oxidizes ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] to produce a radical cation (ABTS<sup>+</sup>). ABTS<sup>+</sup> is a soluble chromagen that can be monitored spectrophotometrically. Antioxidants suppress the formation of the radical cation in a concentration-dependent manner. Trolox is a water-soluble vitamin E analogue that serves as the standard antioxidant. The antioxidant activity relative to the concentration of the Trolox standard was determined for each sample.

### Statistical Analysis

For time to first tumor, Kaplan-Meier estimates of the survival function were generated for both males and females, and the median survival times with 95% confidence intervals (95% CI) were calculated. The log-rank test was used to compare overall survival between genders.

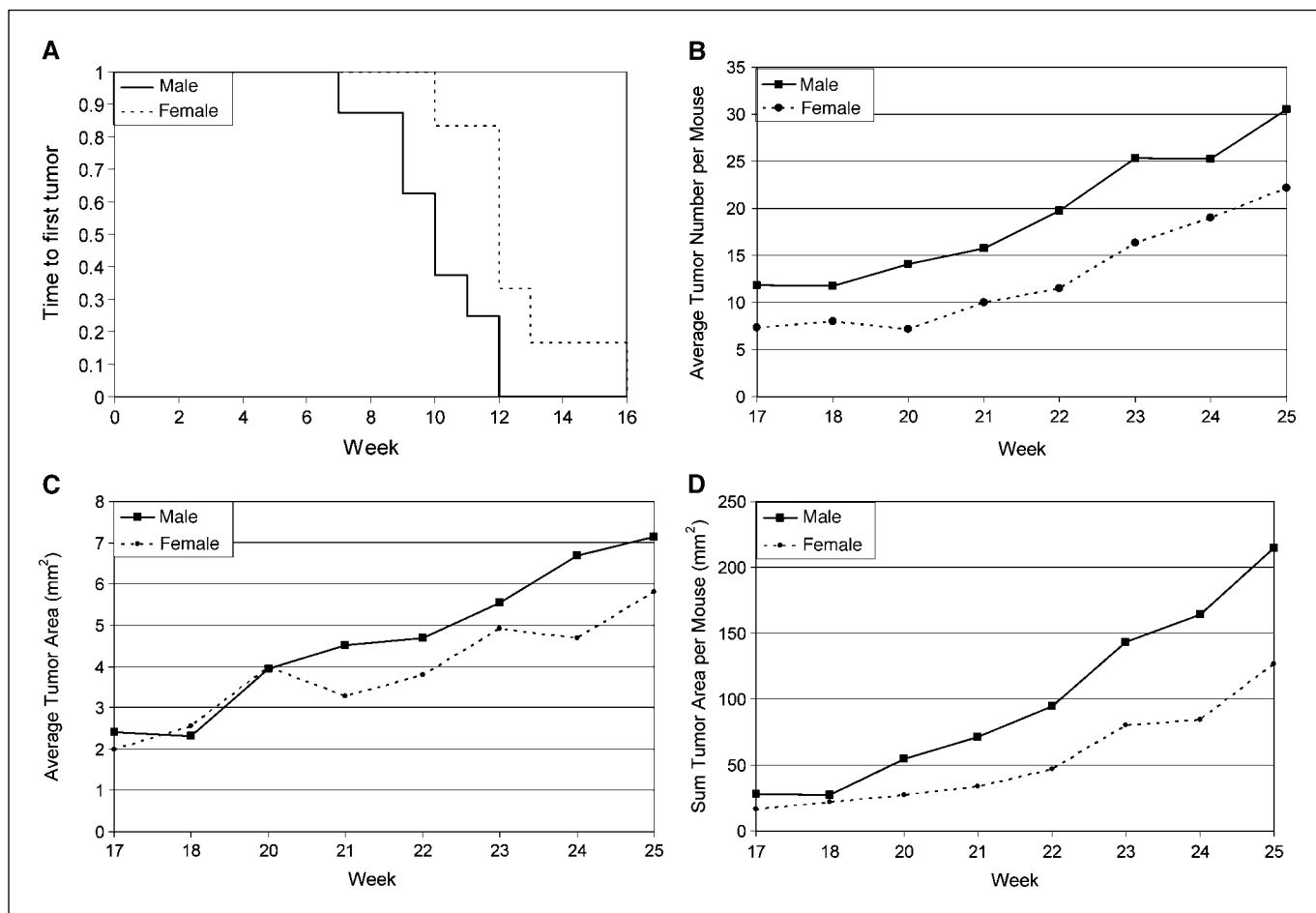
The number of tumors for all weeks after cessation of UVB exposure was modeled using repeated measures Poisson regression. From the model, the estimated weekly increase in the number of tumors as well as the difference in tumor count between genders was estimated with 95% CI. Both the average tumor size and total tumor area were first log-transformed, and then random effects models were applied to the log-transformed data to estimate trends over time as well as differences between genders. Two-sample *t* tests were used to compare skin fold thickness, myeloperoxidase, DNA damage, and antioxidant activity between genders.

For all comparisons, a two-sided  $\alpha = 0.05$  level of significance was used. Tumor data analyses were done using SAS 9.1 (SAS Institute, Inc., Cary, NC). Analyses of skin fold thickness, myeloperoxidase, DNA damage, and antioxidant activity were done using Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA).

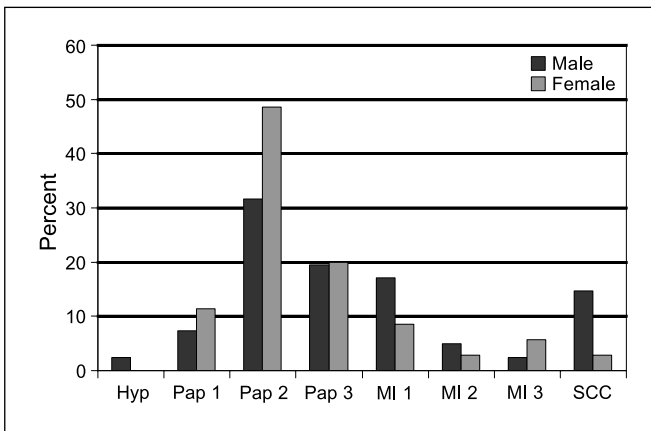
## Results

### Male gender bias in UVB-induced skin carcinogenesis.

Exposing male and female mice to an equal dose of UVB resulted in a significantly worse tumor prognosis for male mice compared with female mice. The time to the first tumor was significantly shorter in the males compared with the females, with a median time of 10 weeks to first tumor in males (95% CI, 9–12%) and 12 weeks in females (95% CI, 12–13%;  $P = 0.0199$ ; Fig. 1A). All mice developed tumors by week 16, after which UVB exposure was stopped.



**Figure 1.** Enhanced UVB-induced skin carcinogenesis in male Skh-1 mice compared with female mice. Male and female Skh-1 mice were exposed to  $2,240 \text{ J/m}^2$  UVB thrice per week for 16 wk, and tumors  $>1 \text{ mm}$  in diameter were assessed weekly from first appearance at week 6 until week 25. A, Kaplan-Meier survival analysis of time to first tumor shows significantly earlier tumor development in male mice compared with female mice. Points, after the cessation of UVB exposure, average tumor number (B), average tumor area (C), and average sum of tumor area (D) per mouse.



**Figure 2.** More histologically advanced tumors on male mice compared with female mice. Male and female Skh-1 mice were exposed to 2,240 J/m<sup>2</sup> for 16 wk and sacrificed for analysis of pathologic grade at week 25. Three tumors per animal were graded in a blinded manner by a board-certified veterinary pathologist. Columns, percentage of tumors per histologic grade per gender.

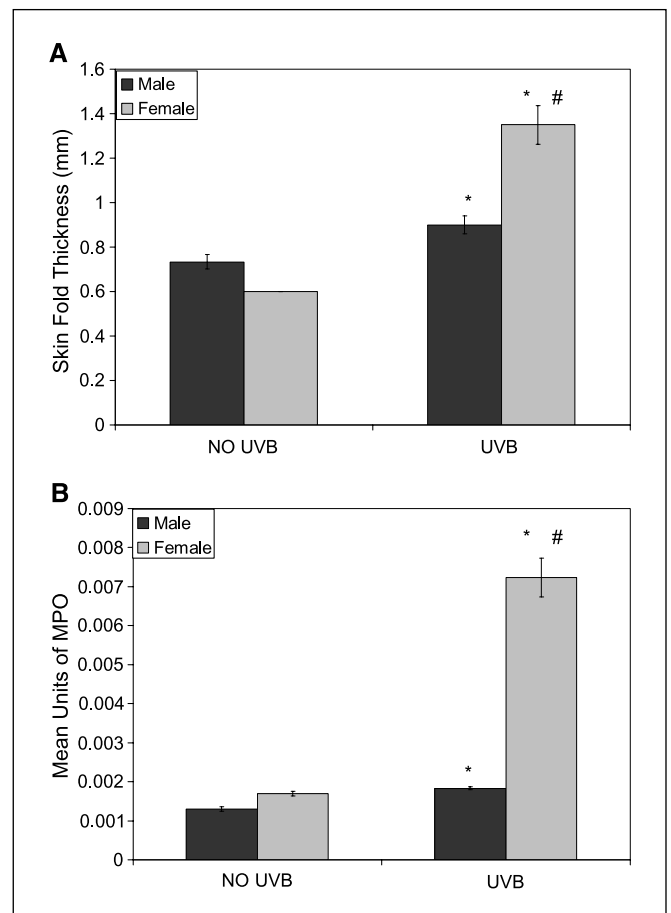
Based on the tumor count model, male mice had ~50% more tumors than the female mice at all time points after the cessation of UVB exposure at week 16 (estimated ratio of male tumors to female tumors = 1.53;  $P = 0.0026$ ; Fig. 1B). In addition, the average tumor count increased by 15% each week for both males and females after the cessation of UVB exposure. For each mouse, both the average tumor size and total tumor area were calculated. Figure 1C shows the average tumor size for both males and females. Note that there was no significant difference in tumor size between males and females at week 17 ( $P = 0.93$ ), 1 week after cessation of UVB exposure. However, by week 25, males had, on average, 43% larger tumors than females ( $P = 0.0592$ ). The rate of tumor growth was 17% per week in males (95% CI, 13–20%), which was significantly greater than the 11% increase per week in females (95% CI, 8–15%;  $P = 0.0367$ ). The total tumor area per mouse is a measure of the total tumor burden per mouse, taking both tumor number and individual tumor area into account (Fig. 1D). Overall, male mice had nearly twice the total tumor area compared with females at all time points after the cessation of UVB exposure (ratio of male tumor area to female tumor area, 1.98;  $P = 0.0177$ ).

Histologic sections from three tumors per UVB-exposed animal were examined for histologic grade (Fig. 2). Of the tumors graded, 38.5% of the male tumors were malignant compared with 18.2% in females. Therefore, 81.8% of the female tumors and 61.5% of the male tumors were benign. Additionally, the percentage of tumors that were fully invasive squamous cell carcinomas in the male mice (14.6%) was greater than in the female mice (2.9%). In both males and females, the grade 2 papilloma was the most commonly diagnosed tumor; however, this tumor type constituted a larger fraction of the tumors in female than in male mice. Overall, male mice had a higher proportion of microinvasive squamous cell carcinomas than female mice. Taken together, these findings suggest that tumor progression was accelerated in male mice compared with female mice. This suggestion is consistent with the trend for larger tumors in the male compared with the female mice.

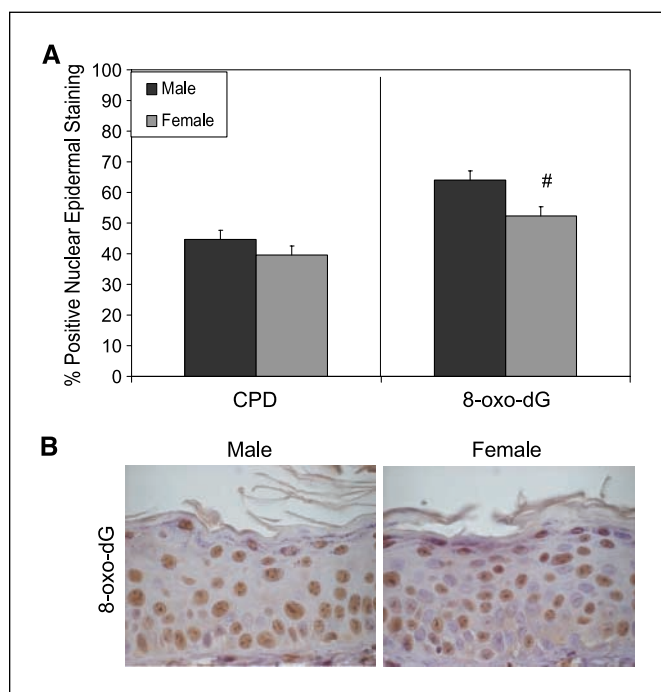
**Effect of a single acute UVB dose on cutaneous inflammation.** Male and female mice were exposed once to 2,240 J/m<sup>2</sup> UVB and sacrificed for analysis 48 h after exposure to evaluate gender differences in the acute cutaneous inflammatory response. The

cutaneous inflammatory response was evaluated by differences in skin fold thickness, as a measure of edema, and myeloperoxidase activity, as a measure of neutrophil infiltration (33). Exposure to this dose of UVB induced a significant change in skin fold thickness from baseline measurements in both males (0.15 mm increase,  $P = 0.007$ ) and females (0.78 mm increase,  $P = 0.0009$ ; Fig. 3A). However, females had a 5-fold greater increase in skin fold thickness compared with males ( $P = 0.00045$ ). Exposure to UVB also induced a significant increase in myeloperoxidase activity compared with unexposed mice in both males ( $P = 0.001$ ) and females ( $P = 0.0004$ ; Fig. 3B). Females exposed to UVB had thrice the myeloperoxidase activity of exposed males ( $P = 0.0004$ ). Neutrophil infiltration patterns in the dermis of these unirradiated and irradiated mice, as determined immunohistochemically, matched the patterns of myeloperoxidase activity (data not shown).

**Acute UVB-induced DNA damage.** DNA damage was assessed by immunohistochemical analysis of two different markers 48 h after exposure: CPDs as a measure of direct UVB-induced DNA damage and 8-oxo-dG adducts as a marker of indirect UVB-induced DNA damage (Fig. 4A). There was no detectable CPD staining in unirradiated skin and a significant increase in percentage of positively staining nuclei in both the male ( $P = 0.00023$ ) and female



**Figure 3.** Gender differences in acute UVB-induced inflammation. Male and female Skh-1 mice were irradiated once with 2,240 J/m<sup>2</sup> UVB and sacrificed for analysis 48 h after exposure. A, skin fold thickness measurements in UVB-exposed and unirradiated control mice. B, mean units of myeloperoxidase (MPO) in UVB-exposed and unirradiated control mice. Columns, average; bars, SE; \*,  $P < 0.05$ , difference to no UVB; #,  $P < 0.05$ , difference to males.



**Figure 4.** Gender differences in acute UVB-induced DNA damage. Male and female Skh-1 mice were irradiated once with 2,240 J/m<sup>2</sup> UVB and sacrificed for analysis 48 h after exposure. Formalin-fixed, paraffin-embedded tissue sections were immunohistochemically stained for CPD or 8-oxo-dG. **A**, percentage positive staining nuclei per total nuclei in the epidermis. Columns, average; bars, SE. #,  $P < 0.05$ , difference to males. Differences between the different types of damage cannot be compared because of the differences in the immunohistochemical processes. **B**, immunohistochemical staining of 8-oxo-dG in male and female UVB exposed epidermis. Original magnification,  $\times 100$ .

( $P = 0.00016$ ) irradiated skin. However, there were no detectable differences in the numbers of CPD-positive cells between male and female ( $P = 0.29$ ) UVB-exposed skin. Interestingly, the percentage of nuclei staining for 8-oxo-dG was significantly higher in unirradiated male skin compared with female skin (50.3% versus 31.7%;  $P = 0.00024$ ). This gender difference held true for UVB-exposed male and female skin (63.9% and 52.3%;  $P = 0.0027$ ). Representative sections of 8-oxo-dG immunohistochemistry on male and female UVB-exposed skin are shown in Fig. 4B.

**Antioxidant activity.** The antioxidant activity assay was conducted to quantitate the cumulative antioxidant capacity of the murine skin (Fig. 5). The antioxidant concentration is represented relative to the concentration of the Trolox standard provided in the kit. Male and female mice were exposed once to 2,240 J/m<sup>2</sup> UVB and sacrificed for analysis 48 h after exposure. In unirradiated skin, females had significantly more antioxidant activity compared with the males ( $P = 0.00017$ ). Following UVB exposure, female skin continued to have significantly more antioxidant activity than the males ( $P = 0.015$ ). However, there was significantly less activity in the UVB-exposed female skin compared with the unirradiated controls ( $P = 0.016$ ). This same trend was seen in the male skin.

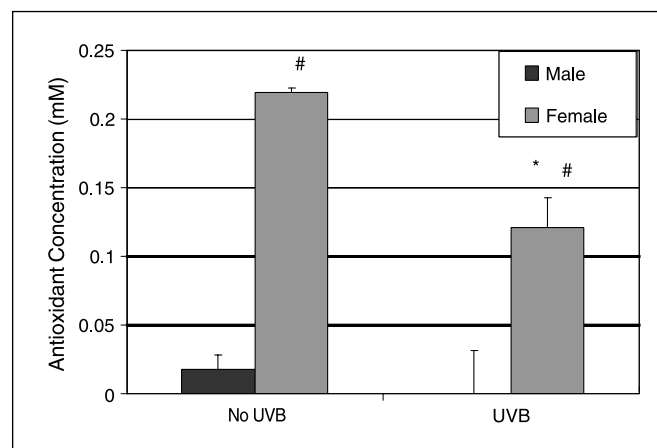
## Discussion

The present study used a well-established animal model of UVB-induced skin carcinogenesis to show clearly, and for the first time, that male mice develop more tumors than female mice when

exposed to equal doses of UVB. These results support the results of the epidemiologic studies to date (1–4).<sup>3</sup> Although males tend to have more sun exposure and are less likely to use protection, which may potentially lead to increased tumor formation (5, 6), our data shows an additional squamous cell carcinoma susceptibility related to the male gender. The fact that this gender difference has been seen both in epidemiologic studies of humans and in our animal model with equal UV exposure suggests that this difference may be due not entirely to sociologic differences but rather to specific biological characteristics. In the current study, male mice developed tumors significantly earlier than the female mice. Male mice developed significantly more tumors than female mice and, by week 25, tended to have larger tumors. Moreover, the total tumor burden in males was nearly twice that in females. In addition to having more tumors and larger tumors, males had tumors of a higher histologic grade than females, suggesting accelerated progression of the tumors. Thus, when receiving identical UVB exposure, male mice have enhanced skin tumor susceptibility.

We expected to see a correlation between the degree of acute UVB-induced inflammation and chronic UVB-induced tumor formation. Previously, our laboratory has shown, using female Skh-1 mice, that the anti-inflammatory effects of celecoxib after acute UVB exposure (15) correlated with reduced UVB-induced tumor formation (19). However, in the current study, males showed less of an inflammatory response, as determined by skin fold thickness and myeloperoxidase activity, compared with females exposed to the same dose, but developed more tumors. Studies by Gambichler et al. (34) showed that the MED in humans is not significantly influenced by gender. Although it appears from our previous studies, as well as the studies of others, that there is a correlation between levels of cutaneous inflammation and skin tumor susceptibility in female mice, this does not appear to hold true in male skin (15–20). Thus, the difference in inflammatory response to UVB did not account for the difference in skin tumor susceptibility between male and female mice in our study.

We therefore investigated if susceptibility to UVB-induced DNA damage or ability to repair this DNA damage influenced the gender difference in the skin tumor susceptibility that we observed.



**Figure 5.** Gender differences in antioxidant capacities. Male and female Skh-1 mice were irradiated once with 2,240 J/m<sup>2</sup> UVB and sacrificed for analysis 48 h after exposure. The antioxidant activity is represented as a concentration relative to the concentration of the Trolox standard. Columns, average; bars, SE. \*,  $P < 0.05$ , difference to no UVB; #,  $P < 0.05$ , difference to males.

Different types and markers of DNA damage were evaluated in the acutely UVB-exposed skin. CPDs are a form of direct UVB damage. Quantitative immunohistochemical staining indicated that there were no significant differences in numbers of CPD-positive nuclei between male and female epidermis. Because the males and females were exposed to equal doses of UV, it is not surprising that there were no differences in the extent of this type of damage. Because our samples were analyzed 48 h after UV and CPDs are formed immediately after exposure, the lack of difference also suggests that there were no gender differences in repair of this type of lesion. Quite interestingly, there was a significant difference in the percentage of 8-oxo-dG-positive cells detected in unirradiated male and female skin. The fact that males had more of this indirect, oxidative damage both in unexposed skin and at 48 h after exposure suggests that males are more susceptible to the formation of 8-oxo-dG adducts or have a less effective oxidative repair capacity, especially in view of the limited inflammatory response in the males. We are not the first to report gender differences in 8-oxo-dG formation. A previous study has shown that males tend to have greater steady-state levels of 8-oxo-dG in the white blood cells of healthy individuals (discussed in ref. 35). Studies on hepatic mitochondrial DNA have shown the levels of 8-oxo-dG to be 4-fold higher in the males compared with the females (36). Interestingly, studies by Paz-Elizur et al. (37) showed a lack of gender differences in OGG1 (8-oxoguanine DNA glycosylase) activity, the enzyme responsible for removing 8-oxo-dG lesions from the DNA. Semiquantitative reverse transcription-PCR analysis of OGG1 mRNA expression in the skin from the unirradiated and acute UVB-exposed skin in this study showed no significant gender difference (data not shown). Although functional differences in OGG1 in male and female skin were not identified in this study, it is one area of focus in our ongoing investigations into the role of gender in skin carcinogenesis.

Gender differences in oxidative damage also suggest differences in the antioxidant pathways designed to remove reactive oxygen species, thus preventing DNA damage. The total antioxidant capacity was determined in the male and female unirradiated and UVB-exposed skin. There were significant gender differences, with the unirradiated and UVB-exposed female skin demonstrating more activity than their male counterparts. UVB exposure significantly decreased the antioxidant activity in the female mice compared with the unirradiated controls. This same trend was seen in the males; however, the results were not significant, likely due to the low baseline activity in the unirradiated males. Katalinic et al. (24) have shown greater antioxidant capacity in female rat heart, kidney, and liver. Other studies have shown that glutathione

peroxidase and manganese superoxide dismutase, two antioxidant enzymes, have greater expression and activity in female mitochondria compared with male mitochondria. Additionally, H<sub>2</sub>O<sub>2</sub> levels are higher in males than females. Glutathione, an intracellular antioxidant, is more abundant in female hepatic mitochondria than in male mitochondria (reviewed in ref. 38). Collectively, these studies showed enhanced antioxidant capacity in females, suggesting a potential biological basis for gender differences in skin carcinogenesis. The present study evaluated the total antioxidant capacity of the skin in male and female mice. To our knowledge, gender differences in specific antioxidant enzymes have not been reported in UVB-exposed mouse skin and are the focus of future studies.

Gender differences in tumor formation suggest a possible role for sex hormones in skin carcinogenesis. Skin is not traditionally considered a sex hormone-responsive tissue; however, studies have shown that such hormones can have a significant influence on the skin. Studies by Azzi et al. (39) showed gender differences in mouse skin morphology, an effect of gonadectomy on skin structure, and an influence of exogenous estrogen and estrogen precursors on skin histology. Postmenopausal women with diminished systemic estrogen production show significant changes in the skin, including delayed wound healing, skin thinning, and increased wrinkling, all of which can be reversed with hormone replacement therapy (40). Studies by Ashcroft et al. (41) have shown enhanced wound healing in both males and females with topical application of 17 $\beta$ -estradiol, further supporting the potential influence of estrogen in the skin. Studies on hepatic mitochondrial antioxidant capacities showed that estrogen acted via estrogen receptors to reduce H<sub>2</sub>O<sub>2</sub> production and enhance antioxidant enzyme expression (36). Studies in our laboratory are currently under way to examine the influence of exogenous estrogen on UVB-induced cutaneous inflammation and skin carcinogenesis; these studies will further investigate the hormonal component of gender differences in tumor development. Very little is yet known regarding the role of gender in cutaneous responses to UVB exposure; thus, this report is the first step in the systematic examination of gender differences in skin carcinogenesis.

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