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Original Article

Passive cigarette smoke exposure inhibits ultraviolet light B-induced skin tumors in SKH-1 hairless mice by blocking the nuclear factor kappa B signalling pathway

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Abstract: Chronic exposure to sunlight [ultraviolet light B (UVB) irradiation] is the most common cause of non-melanoma skin tumors. In the present study, we investigated the effects of passive cigarette smoke superimposed over UVB irradiation, on tumor development, skin pathology and matrix changes in SKH-1 hairless mice. Groups of mice were exposed to 0.1 J/cm^2 of UVB five times per week for 20 weeks and/or exposure to passive cigarette smoke from 40 cigarettes a day over the same time period. UVB exposure resulted in an average of four large squamous cell carcinomas (SCC) and 15 smaller papillomas per mouse, whereas exposing the mice to both UVB + passive cigarette smoke completely prevented SCC formation and averaged less than one small papilloma per mouse. Oxidative DNA damage was investigated and there were no significant changes in the levels of urinary DNA adducts between control, smoke, UV and UV + smoke groups with the exception of 8-oxo guanine which was significantly reduced in the presence of passive cigarette smoke. Immunohistochemistry results revealed that tumor necrosis factor receptor 2 (TNF-R2), glycogen synthase kinase-3 beta, nuclear factor kappa B (NF-κB)/p65, KI-67 and cyclooxygenase 2 (COX-2) were markedly up-regulated in the epithelium by UVB exposure, whereas passive smoke exposure combined with the UVB irradiation completely blocked the expression of these proteins. Our results suggest that passive smoke exposure prevents UVB-induced SCC in mice and dramatically reduces the incidence of non-malignant papillomas by altering the NF-κB signalling pathway of tumorogenesis.

Key words: contact hypersensitivity – nuclear factor kappa B – passive cigarette smoke – skin tumor – ultraviolet light B

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Introduction

Each year there is a small but significant increase in the amount of ultraviolet radiation (UVR) that is reaching the earth's surface as a result of a reduction in the ozone layer in our atmosphere. UVR is known to initiate cell stress, activate transcription factors and induce changes in gene expression through intracellular signal transduction pathways, all of which may support skin cancer. Basal and squamous cell carcinomas (SCC) are the most common forms of skin cancer, accounting for nearly half of all cancers in the United States and it has been well established that it is exposure to the ultraviolet light B (UVB) component of sunlight that is the major etiologic event for the initiation of these tumors (1). UVB radiation is a complete carcinogen, being able to initiate, promote and advance the development of skin cancer (2).

At the molecular level, UVB can cause DNA damage, particularly cyclobutane pyrimidine dimers (CPD) and (6-4) photoproducts which induce mutation in the epidermal cells leading to gene mutations and the development of cancer cells (3–5). DNA damage is usually repaired by nucleotide excision repair enzymes; however, chronic UV exposure and insufficient repair, followed by errors in replication, can lead to mutations that represent initiation events in carcinogenesis. During tumor initiation, UV irradiation can also cause chromosomal alternations and mutations via direct DNA damage and/or production of reactive oxygen species

Abbreviations: UVB, ultraviolet light B; NF-κB, nuclear factor kappa B; CHS, contact hypersensitivity; ROS, reactive oxygen species.

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(ROS) (2,6). ROS resulting from UV irradiation are known to activate many transcription factors such as those found in the activator protein (AP-1) and nuclear factor kappa B (NF-κB) pathways which may contribute to cell proliferation and/or apoptotic cell death.

Another environmental factor that is known to be a major risk factor in cancer development is cigarette smoke exposure. Cigarette smoke is a complex chemical mixture containing thousands of different compounds, of which over 100 are known carcinogens, co-carcinogens, mutagens or tumor promoters (7). In recent years, there has been a special emphasis placed upon the relevance of environmental or passive cigarette smoke and carcinogenesis (8). Environmental cigarette smoke contains not only potential carcinogens, such as benzo [a] pyrene and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), but also a large amount of oxygen radical forming substances, such as catechol and hydroquinone which are known to disturb biological systems by reacting with a variety of their constitute molecules (9).

Epidemiological studies as well as laboratory research have established a definite link between cigarette smoke exposure and tumor development (10). There have been no studies, however, showing the effects of passive cigarette exposure superimposed on skin that had previously been exposed to chronic UVB irradiation. Would this enhance the carcinogenic potential of both exposures, or would there be no additive effect? In the following series of experiments, we show the effects of passive cigarette smoke superimposed on UVB irradiation in terms of tumor development and skin matrix changes.

Materials and methods

Exposure to UVB irradiation and passive cigarette smoke

All animal research was approved by the University of Texas Health Center at Tyler Animal Research Committee. For the first experiment, 80 female SKH-1 hairless mice, 6 weeks of age, were purchased from Charles Rivers Laboratories, Wilmington, MA. All mice were housed in the University of Texas Health Center at Tyler Vivarium on a 12-h light/dark cycle at 25°C. UVB irradiation was administered with a 400-W high-pressure mercury halide lamp equipped with a UVB filter (Dermalight Systems, Inc. of Sherman Oaks, CA, USA). This source approaches the sun spectrum of 90% UVA and 10% UVB. The UV-exposed mice received 0.1 J/cm^2 of UVB (20 s) irradiation per treatment 5 days a week for 22 weeks with the lamp positioned 20 cm above the mice. The cumulative UVB dose administered in the experiment was 11.0 J/cm^2. The UV + smoke group received the same UVB exposure, followed immediately with passive cigarette smoke exposure. Passive cigarette smoke exposure was administered using a TE-10c microprocessor controlled cigarette smoking machine (Teague Enterprises, Davis, CA, USA). The research cigarettes used were 2R4F low tar modern cigarettes (Kentucky Tobacco Research and Development Center University of Kentucky, Lexington, KY, USA). The smoke generated was a mixture of mainstream smoke (11%) and sidestream smoke (89%). The experimental mice were exposed to three cigarettes at a time for a total of 40 cigarettes per day. Total exposure time was approximately 2 h each day 5 days a week for 22 weeks. Chamber particulate concentration (TSP) was 250 mg/mm^3. Mice receiving the lower dose of smoke exposure received one cigarette at a time for a total of 12 cigarettes per day and a TSP of 89 mg/mm^3. Control mice and mice receiving only UVB were placed in cages under similar conditions as the smoked animals for 2 h but were not exposed to cigarette smoke.

Contact hypersensitivity/immune suppression

In a separate experiment, 40 female SKH-1 mice were used to determine the effect of passive cigarette smoke exposure, alone or in combination with UVB irradiation, on the cutaneous immunologic response. Forty female SKH-1 hairless mice were divided into 10 mice per group. The mice were immunized by painting 20 μl of a solution of acetone/corn oil (4:1) containing 1% picric chloride (oxazolone) solution on the stomach of each mouse. Five days after immunization, all groups were challenged with 10 μl of a 0.5% picric chloride solution applied to each side of one ear. After 24 h, ear swelling was measured with a digital micrometer as a measure of contact hypersensitivity (CHS) response. The thickness of the non-challenged ear was subtracted from the thickness of the challenged ear to obtain a net ear swelling value, which represents the hypersensitivity response. No attempt was made to protect the ears during UV irradiation.

Tumor count

Pictures were taken with an Olympus C-4000 digital camera (Olympus Optical Co., Ltd., Melville, NY, USA) of SKH-1 hairless mice in all four groups at various time points to document tumor formation and changes in skin appearance. At the end of the experiment (22 weeks), tumors on the exposed backs of each mouse were categorized as either 2 mm or larger (SCC) or small non-cancerous papillomas of less than 2 mm in diameter. All larger tumors were later confirmed as SCC by histology. Measurements were made prior to killing the animals in order to visualize the smaller papillomas.

Matrix changes

A 3-mm biopsy punch of the skin was hydrolyzed and assayed for desmosine (elastin) and hydroxyproline (collagen) as described previously (11).

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Histology and immunohistochemistry

Histological studies were restricted to the keratinocyte layer as this is the region of interest in terms of skin tumor development. Twenty-two weeks after the start of the experiment, the mice were killed and a section of skin (3×4 cm) was removed from the back of each mouse and fixed in Excell fixative solution (American Master Tech Scientific Inc-Lodi, CA, USA) for 24 h. A small rectangle of skin was removed and oriented parallel to the body axis, paraffin embedded and 5 μm sagittal sections removed for histology. Antigen retrieval was performed in 0.01% sodium citrate buffer (pH 6.0) at 95°C for 5 min. Primary antibodies TNF-2R, NF-κB, KI-67, COX-2, glycogen synthase kinase-3 (GSK-3) and the HRP staining kit were obtained from Lab Vision Corporation, Fremont, CA. Apoptosis was measured using the Tunnel assay (Promega, WI, USA). Anti mouse von Willebrand antibody was obtained from Sigma Chemical Company, St Louis, MO. All histology was viewed and photographed with an Olympus BX50 light microscope fitted with an Olympus DP12digital camera (Olympus Optical Co., Ltd.).

Oxidative damage to DNA

Daily urine samples obtained from each group were pooled each week during the 22-week experiment and stored frozen at −20°C until assayed. The levels of 8-oxodG, 8-oxogua and 8-oxoguo were measured by using the HPLC-electrospray tandem mass spectrometry essentially as described previously (12,13). Detection limits were approximately 0.3 nM. Values were corrected for urine concentration using urine creatinine as the denominator.

Statistics

Statistical analysis was performed using a computer software package (INSTAT, GRAPHPAD, San Diego, CA). Significance was set at $P < 0.05$.

Results

Effect of cigarette smoke exposure on UVB-induced tumor development

On week 14, after 7.0 J/cm^2 of cumulative UVB radiation, small papillomas appeared on the UV irradiated mice and rapidly increased in size and number. By the end of 22 weeks, the backs of all the UV mice were covered with an average of four SCC and 15 papillomas as a result of the cumulative dose of 11.0 J/cm^2 UVB (Fig. 1b). All of the larger tumors examined were classified histologically as SCC characterized by Hyperkeratosis, with a proliferation of atypical keratinocytes and characteristic keratin pearls. Surprisingly, mice exposed to 22 weeks of UVB, followed by exposure to the smoke from 40 cigarettes per day showed no evidence of SCC (Fig. 1c) and averaged only one small papilloma per mouse. No mice in either the SKH-1 hairless control group or mice exposed to smoke alone developed tumors by the end of 22 weeks (Figs 1a and d). Exposure of the SKH-1 hairless mice to UVB for 22 weeks resulted in significant changes in the appearance of the skin other than tumors. There was a pronounced thickening and wrinkling of the skin, particularly around the shoulder and neck areas (Fig. 1b). Superimposing cigarette smoke on the UVB exposure noticeable attenuated the wrinkled and thickened appearance, with the skin almost appearing normal (Fig. 1c). Smoke exposure alone (Fig. 1d) resulted in very thin, fragile skin that tended to sag beneath the mice, stretching from the top of the hind legs to the front legs. There was also a small reduction in body weight gains.

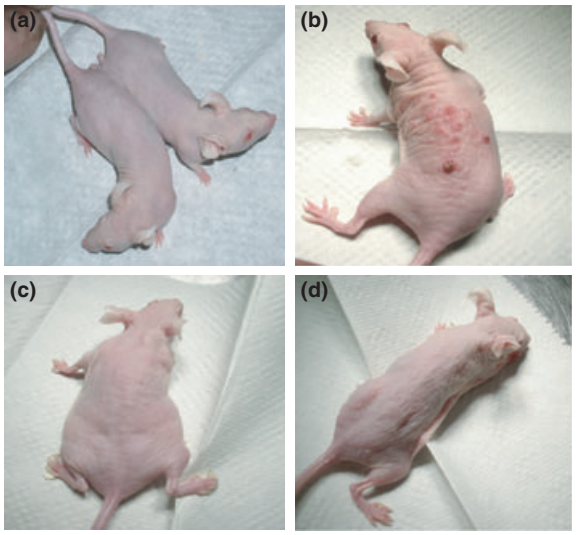

Figure 1. Skin tumors following exposure to UVB irradiation and passive cigarette smoke. The skin of control mice (a) is smooth with no evidence of tumors, while the skin of UV-exposed mice (b) is covered with papillomas and larger SCC. Mice exposed to UVB followed by passive smoke exposure (c) show no SCC and are virtually free from papillomas as are the mice exposed to passive cigarette smoke alone (d).

A second experiment was conducted with the same exposure groups to confirm the passive cigarette smoke effect with the addition of another group of mice receiving the same dose of UVB but exposed to smoke from only 12 cigarettes over a 1 h time period. Again, we observed multiple SCC on the backs of the UVB irradiated mice and none on the control, smoke alone or UVB + smoke from 40 cigarettes. However, there was a significant lack of tumor protection in the mice exposed to UVB and 12 cigarettes over 1 h. These mice showed an average of three SCC and 10 papillomas which is only slightly less than

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mice receiving UVB alone. A summary of the tumor counts for the different experiments is shown in Table 1.

Matrix composition

The content of elastin in the skin from the mice in each experimental group was calculated by quantitating the amount of desmosine per 3-mm biopsy punch. UVB irradiation produced the expected increase in desmosine, reflecting an increase in size and number of skin elastic fibres as has been shown previously (14). Desmosines increased significantly from an average of 149 ± 7 picomole desmosine per 2-mm punch in the control mice to 201 ± 19 picomole ($P < 0.001$) in the UVB-exposed mice. Exposure to passive cigarette smoke superimposed on the UVB exposure partially prevented the elastin increase, lowering the desmosines to 173 ± 16 picomole ($P < 0.005$). Cigarette smoke alone had no effect on the skin elastin content (146 ± 12 picomole) ($P < 0.62$). Collagen content, expressed as the amount of hydroxyproline per 2-mm skin biopsy punch in the control mice 450 ± 31 nmole, was not significantly different from the UVB (425 ± 78 nmole) ($P < 0.36$), UVB + smoke (456 ± 54 nmole) ($P < 0.75$) or the smoke alone (480 ± 58 nmole) ($P < 0.18$). All values are the mean of 10 animals with standard deviation.

There was an influx of inflammatory cells, both histocytes and neutrophils, in the dermis of the UVB irradiated skin after 22 weeks but we saw no evidence that passive cigarette exposure altered the inflammatory cell load. We did not follow the inflammatory cell influx as a function of time after exposure or days on the experiment.

Contact hypersensitivity

SKH-1 hairless mice immunized with 1% picric chloride solution and subsequently challenged on the ears with 0.5% picric chloride solution demonstrated a significant CHS response as evidenced by an inflammatory cell influx and defined by ear swelling (Fig. 2). UVB irradiation prior to immunization suppressed the induction of the CHS response. This was true also for the mice exposed to cigarette smoke. The combination of UVB + smoke was just as effective in blocking the immune response as UVB alone.

Table 1. Tumor count of mice

Group	UV	UV + smoke	UV + 12 cigarettes
Tumors	57	0	27
Average	3.3 ± 1.5	0	2.7 ± 2.1**
Papillomas	285	22	81
Average	16.8 ± 9.0	1.1 ± 1.3*	8.1 ± 4.8*
Incidence	17/17	9/19	8/10

Average values with SD.
*$P < 0.001$ compared with the UV group.
**$P < 0.5$ compared with the UV group.

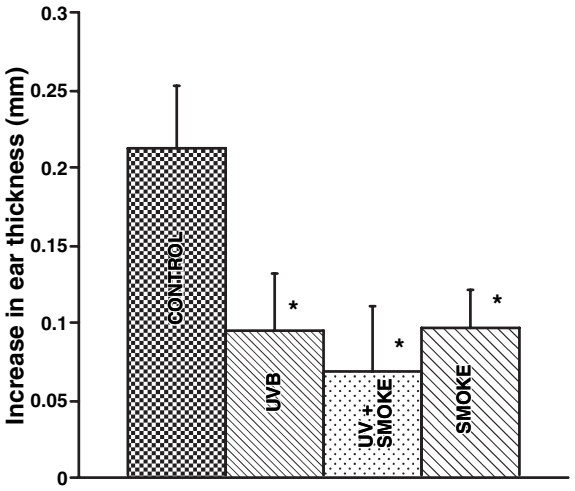

Figure 2. Contact hypersensitivity response to 1% picric chloride solution. Mean ear swelling was measured in response to immunization with picric chloride followed by treatment with picric chloride 5 days later. Ear swelling was assessed after 24 h. Immune response was significantly inhibited with 1.0 J/cm^2 of UVB as well as exposure to passive cigarette smoke. Each value represents the mean ± SD of 10 SKH-1 hairless mice. Asterisk (*) indicates significantly different from control ($P < 0.001$).

Oxidative damage in DNA

In the ROS experiments, we investigated guanosine and all five major bases and nucleoside adducts that may be present in the urine and which may reflect oxidative damage and potential causes of skin tumors. For most of the adducts measured, there were no significant differences between groups and the data is not shown. Figure 3 shows the effect of UVB and passive cigarette smoke on the urine levels of 8-oxoguanine. Control urine and urine from mice exposed to UVB varied from week to week but both showed about the same level of 8-oxoguanine during the first 10 weeks of the experiment. Urine from mice exposed to passive smoke, whether in combination with UV or by itself, showed significantly lower levels of 8-oxoguanine. The reduction in 8-oxoguanine occurred early and was consistent throughout the 10-week period.

Immunohistochemistry

Immunohistochemistry was performed on skin sections from the various groups of mice to detect alterations in the amount of proteins implicated in the NF-κB signalling pathway (Fig. 4). Antigens assayed for are shown on the left with representative skin sections from the different

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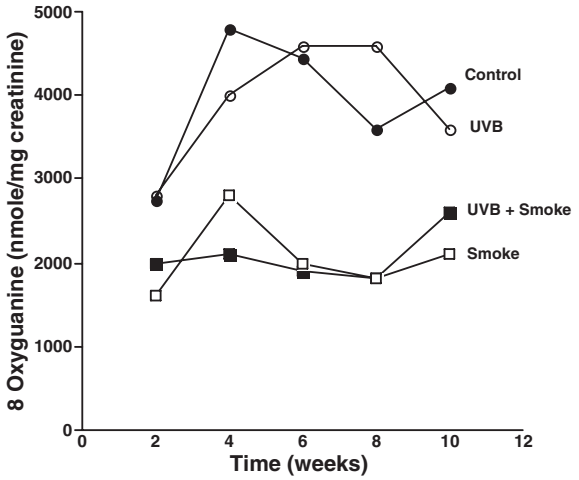

Figure 3. Measurement of oxidative damage. 8-Oxoguanine levels were measured with using HPLC-electrospray tandem mass spectrometry. Smoke exposure in the presence or absence of UVB consistently reduced the urinary levels of the DNA adduct during the first 10 weeks. Each value represents a pool of weekly urine from 10 mice per group.

experimental groups illustrated on the right. TNF-R2 was detected in low levels in the epidermis (keratinocytes) of control skin and the skin of mice exposed to passive cigarette smoke alone. We found a very high level of TNF-R2 expression in the UVB irradiated skin, whereas exposure to passive cigarette smoke superimposed on the UVB treatment completely prevented the increase of TNF-R2 expression observed in the UV irradiated mice. Very similar results were found for all the signalling factors in the NF-κB pathway that we measured. Very low activity of GSK3-beta, NF-κB/p65, KI-67 and COX-2 was observed in the control and passive smoke-exposed epithelium; whereas there was a striking elevation in immunoreactivity for these proteins in the epithelium of the UVB treated mice. Even more remarkable was the complete down-regulation of these proteins when the UVB treated mice were exposed to passive cigarette smoke. A high number of apoptotic cells as indicated by a positive TUNEL reaction was observed in UVB and UV + smoke-exposed skin. Control skin showed a few apoptotic cells, whereas skin from mice exposed to passive cigarette smoke showed a relative high number of apoptotic cells considering the thinness of the epithelium.

Angiogenesis associated with tumor development is illustrated in Fig. 5. A typical tumor from a mouse exposed to UVB irradiation and stained with trichrome demonstrates the thickened epidermis (bright red colour) and dysplastic epithelium (yellow), characteristic of SCC. An adjacent section immunostained with an antibody to von Willebrand factor is shown in Fig. 5b. Marked angiogenesis (red) can be seen as a capillary bed immediately below the tumor.

Discussion

The results of the present study confirm that exposure of SKH-1 hairless mice to UVB irradiation for a period of 22 weeks produces multiple SCC and papillomas along the exposed skin surface. In addition, we observed that exposure to high levels of passive cigarette smoke completely prevented the formation of the UVB-induced SCC and dramatically lowered the incidence of papilloma formation. To understand how this could occur, we investigated several pathways by which UVB exposure can cooperatively or independently contribute to skin cancer.

Oxidative damage to cellular biomolecules, such as DNA, is known to play an important role in carcinogenesis. In our experiments, we did not see the anticipated UVB initiated increase in urinary DNA adducts. The only significant change occurred with 8-oxoguanine, which was consistently reduced two fold in the presence of passive cigarette smoke. The mechanism of how cigarette exposure reduced the urinary levels of 8-oxoguanine is uncertain. While urinary excretion of 8-oxoguanine is increased about 50% in human smokers, nothing is known about the effect of environmental smoke exposure on the excretion of DNA adducts. Genes involved in repair of oxidative DNA damage have been shown to be highly expressed in the skin, when exposed to the combination of both smoke and UVB (15). Smoke-induced up-regulation of the complementary DNA repair system in the skin would seemingly make it less susceptible to UVB irradiation-induced tumor formation. This may be one factor involved in our observations of the inhibitory effect of passive cigarette smoke on UVB-induced tumors. Increased urinary excretion of 8-oxoguanine is compatible with a shift between different repair pathways away from the glycosylate pathway, but could also result from increased apoptosis. It has been demonstrated that a shift between different DNA repair pathways can direct cells towards apoptosis/aging and away from the carcinogenic route (16).

Ultraviolet light B is known to suppress the dendritic cell-dependent T cell-mediated cell immune response and induce tolerance to antigens, which can have a pronounced effect on an animal's ability to modulate tumor growth and development (17). CHS experiments, such as we performed in this study, are designed to measure systemic immunosuppression and does not take into account other arms of the immune system. As expected, we found that UVB exposure inhibited the immune response, agreeing with the supposition that tumors have a better chance to develop in immunosuppressed animals. However, it was also apparent that exposure to cigarette smoke, either alone or in combination with UV irradiation, resulted in the same degree of immunosuppression. This suggests that the inhibitory effect of passive cigarette smoke on

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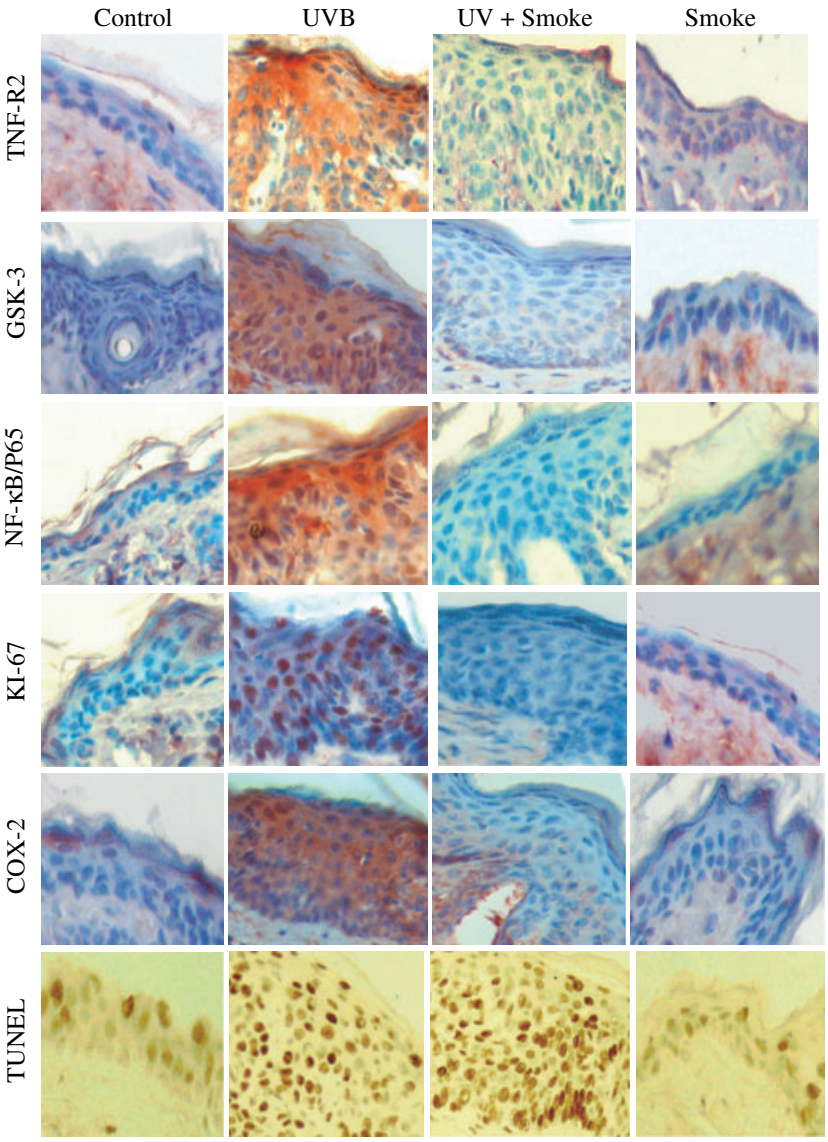

Figure 4. Immunohistochemistry. Paraffin embedded skin (epidermis) tissue sections of control, UVB exposure, UV + smoke and smoke exposure alone. Antibodies were directed against TNF-R2, GSK3-b, NF-κB/P65, KI-67 and COX-2. Images were from 5 μm sagittal sections after 22 weeks of the experiment. Positive reactions are indicated by a red colour contrasted with a blue counter stain. For the tunnel assay apoptotic cells are illustrated by the red-brown nuclei in the epidermal layer of the skin.

UVB-induced tumor development was not related to the immunosuppressive effects of UVB exposure.

Another potential mechanism for the action of passive cigarette smoke on UVB-induced tumors is a direct or indirect effect on signalling factors in the NF-κB pathway. UVB irradiation has been shown to up-regulate the level of tumor necrosis factor alpha (TNF-α) (18). Keratinocytes are activated by UVB to release TNF, and the effects of this potent cytokine are mediated by TNF-R1 and TNF-R2 to activate the NF-κB pathway, promoting subsequent tumor formation (19,20). It is well known that activation of NF-κB plays a critical role in carcinogenesis (21,22). We looked first at the level of TNF-R2 and found, as expected, that UVB irradiation markedly up-regulated this receptor. Exposure to cigarette smoke completely prevented this increase. We next looked at glycogen synthase kinase-3 beta which is activated through TNF signalling. GSK-3 beta is a serine/threonine protein kinase that regulates NF-κB activity through beta-catenin (23). We found that again, there was an increase because of UVB exposure and this increase was blocked by cigarette smoke exposure. NF-κB/p65 expression was strikingly up-regulated by exposure to UVB and was just as strikingly inhibited by exposure to cigarette smoke. At this point in the NF-κB pathway the NF-κB complex is acted upon by I-Kappa-B (IKB) kinases and is then transported into the nucleus where it acts as a transcription factor for many genes. One of these is KI-67, which is a marker for cell proliferation and is known to be up-regulated during tumor development (24). We found a marked increase in KI-67 signalling following UVB

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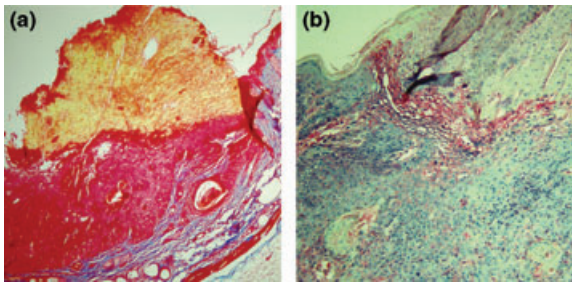

Figure 5. Angiogenesis in a UVB generated squamous cell carcinoma. Tumor tissue section of UV-induced squamous cell carcinoma skin stained with Gomories trichrome (a). Notice thickened epidermis (bright red colour) and dysplastic epithelium (yellow). An adjacent tumor section, immunostained with von Willebrand factor, is illustrated in (b) showing extensive capillary formation under the tumor (red staining).

irradiation indicating a major increase in cell proliferation. As one would predict from our previous observations, exposure to cigarette smoke completely blocked the cell proliferation response. A downstream target enzyme, regulated by NF-κB, is cyclooxygenase-2 (COX-2), which has been shown to be up-regulated in UV-induced skin cancer, whereas inhibition of COX-2 leads to tumor suppression (25). Our experiments confirmed these observations, showing a marked increase in epidermal COX-2 levels following UVB irradiation. Exposure to passive cigarette smoke completely blocked the up-regulation of COX-2.

Angiogenesis is an important factor in tumor growth and we were able to illustrate new capillary growth around UVB-induced SCC. However, as there were no SCC in the UVB + smoke mice to compare with, we were unable to determine the importance of angiogenesis in our passive smoke model of tumor suppression.

Programmed cell death is another factor to consider as there have been several studies showing changes in apoptosis in both UVB and cigarette exposure. Infant monkey lungs exposed to environmental tobacco smoke inhibits NF-κB activity and increases apoptosis (26). Exposure to cigarette smoke can increase apoptosis in the rat gastric mucosa (27), human gingival epithelium (28) and in human umbilical vein endothelial cells through a ROS (29). Irradiation with UVB is also known to cause the formation of apoptotic cells in keratinocytes (30). UVB induces apoptosis in skin cells and at the same time favours their proliferation. Our results agreed well with the literature and indicated that both UVB and passive cigarette smoke exposure increase the incidence of apoptotic cells in the epithelial layer and suggests that apoptosis does not have an important role in the passive smoke inhibition of UV-induced tumors.

Matrix changes were as one would expect. UVB increased overall skin thickness and increased the amount of elastin in the dermis. Cigarette smoke tended to marginalize the UVB effects and this again may be due to inhibition of various transcription factors, cytokines or growth factors. The extracellular matrix has only recently been recognized as having an important role in growth factor utilization.

We found that the lower dose of cigarette smoke exposure was much less effective than the higher dose in preventing UVB-induced tumors. This suggests there is a dose response of cigarette smoke for inhibition of tumor induction. The high level of exposure that we used would be equivalent to what you might expect in a very smoky bar room. It is probable that exposure to low levels of environmental cigarette smoke would not effect the outcome of UVB-induced tumors.

One unanswered question was which cells of the epidermis were responsible for the tumors. The most convincing studies to date suggest they are epidermal stem cells, located in the interfollicular epidermis (31–33) or bulge stem cells in the hair follicle (32,34,35). A recent study by Faurschou et al. (36), using laser surgery to remove the epidermis, suggests that hair follicle bulge cells are the primary source of UV-induced SCC in mouse skin. Although we could not determine in our model if bulge cells had moved to the basal layer of the epidermis, the histological location of our tumors suggested that the tumors arose from the basal layer and had no anatomical association with the hair follicles (Fig. 5).

In conclusion, our studies show that exposure to high concentrations of passive cigarette smokes will block the induction of UVB-induced SCC and papillomas. At least two systems appear to be involved in the observed smoke effect; DNA repair and the NF-κB signalling pathway. The evidence strongly suggests that blocking the NF-κB pathway of signal transduction by some element(s) in passive cigarette smoke is the major cause for the inhibition of UVB-induced tumors in these studies.

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