

Inhibitory Effects of Sodium Salicylate and Acetylsalicylic Acid on UVB-induced Mouse Skin Carcinogenesis¹

Warner B. Bair III, Nancy Hart, Janine Einspahr, Guangming Liu, Zigang Dong, David Alberts, and G. Tim Bowden²

Department of Radiation Oncology [W. B. B., G. T. B.], Department of Medicine [N. H., J. E., D. A.], The University of Arizona Health Sciences Center, Tucson, Arizona 85724, and the Hormel Institute, University of Minnesota, Austin, Minnesota 55912 [G. L., Z. D.]

Abstract

We conducted an *in vivo* carcinogenesis experiment to determine the efficacy of topical aspirin and sodium salicylate (NAS) in preventing UVB-induced nonmelanoma skin cancer. Hairless SKH-1 mice were randomly divided into eight treatment groups. They were treated topically with either 40 or 10 μmol aspirin or NAS three times weekly before 9 kJ/m^2 UVB irradiation. The experiment was carried out over 25 weeks. Both dose levels of NAS significantly inhibited ($P < 0.05$) the rate of tumor formation when compared with vehicle control. The 40 μmol dose of aspirin significantly inhibited the rate of tumor formation ($P < 0.05$), whereas the 10 μmol dose had no inhibitory effect when compared with the vehicle control. To investigate the mechanism of this inhibition, we studied UVB-induced thymine dimer formation in the epidermis of the mouse skin. We found that NAS inhibited UVB-induced thymine dimer formation ($P = 0.0001$), whereas aspirin did not. Therefore, we conclude that NAS prevents UVB-induced tumor growth and formation through a sunscreen effect; whereas, the moderate inhibition of aspirin may be because of a molecular event, such as the inhibition of various UVB signaling pathways.

Introduction

UV exposure is responsible for ~90% of human nonmelanoma skin cancer (1). The UV light that reaches the earth is composed of ~90% UVA (320–400 nm) and 10% UVB (280–320 nm; Ref. 2). Both UVA and UVB are being studied for their skin cancer-causing potential, but currently UVB is thought to be the more important cancer-causing agent. UVB is a complete carcinogen, being able to initiate, promote, and progress the development of skin cancer (3). During initiation, UVB can cause

chromosomal alterations and mutations via direct DNA damage and/or production of reactive oxygen species (4, 5). Tumor promotion occurs via epigenetic effects, such as altered gene expression (4, 6–9), cell membrane damage (8), apoptosis (10, 11), and a compromised immune system (7, 12, 13). Tumor progression appears to involve additional genetic alterations including chromosomal alterations (14).

The initiation effect of UV light is most likely achieved by DNA absorption of UV energy (15). This energy absorption results in the formation of chemical alterations in DNA known as DNA photoproducts. The two major types of photoproducts formed are cyclobutane pyrimidine dimer and (6–4) photoproducts. Both types are formed at dipyrimidine sites in a single DNA strand and are a result of chemical energy being absorbed in chemical bonds between adjacent pyrimidine bases. Cyclobutane pyrimidine dimer and (6–4) photoproducts can occur between cytosine bases as well as cytosine and thymine bases, but most commonly occur between two adjacent thymine bases. This damage occurs immediately on UV irradiation, and then declines steadily as cellular DNA excision repair mechanisms remove and restore the DNA.

UV may act as a promoting agent first at the cell membrane through the activation of the epidermal growth factor receptor (16) or activation of acidic sphingomyelinase (17) and atypical protein kinase C (18). The UV signal is transduced from the plasma membrane via a phosphorylation cascade to the AP-1 transcription factor (8, 19). AP-1³ is an oncogenic transcription factor made up of Jun and Fos family members. It is a known regulator of gene products involved in skin tumor promotion (20) as well as skin tumor malignant progression (21), and AP-1 is found in increasing levels in skin tumors (22, 23). Transformation and progression of skin tumors can be inhibited by AP-1-selective inhibitory drugs (such as retinoids) or by c-jun and c-fos antisense oligonucleotides, or dominant-negative mutants (21, 24, 25). Blocking AP-1 during tumor promotion inhibits tumor formation (26). AP-1 is activated and induced by cancer-causing agents such as UV radiation (UVA, UVB, and UVC; Ref. 19), tumor promoters (21, 23, 26), growth factors (24), and transforming oncogenes. UVB irradiation has been shown to induce c-fos mRNA (4, 9), and protein levels, AP-1 DNA binding, and transactivation in human keratinocytes cells. Inhibition of AP-1 is one of the mechanisms by which chemopreventive agents may block tumor promotion.

Acetylsalicylic acid (aspirin) is one of the most used NSAIDs or salicylates. Since 1892 aspirin and other NSAIDs have been used for their anti-inflammatory and analgesic effects. In recent years, they have been studied for other systemic effects, such as reduction of heart attacks and strokes (27). They

Received 11/12/01; revised 8/9/02; accepted 9/24/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work supported by NIH Grants CA 23074 and CA 27502.

² To whom requests for reprints should be addressed, at Department of Radiation Oncology, The University of Arizona Health Sciences Center, Tucson, AZ 85724. Phone: (520) 626-6006; Fax: (520) 626-4979; E-mail: tbowden@azcc.arizona.edu.

³ The abbreviations used are: AP-1, activator protein 1; COX, cyclooxygenase; NAS, sodium salicylate; NSAID, nonsteroidal anti-inflammatory drug; OMC, octyl 4-methoxycinnamate; IL, interleukin; UVA, ultraviolet light A; MOM, mouse-on-mouse; UVB, ultraviolet light B; UVC, ultraviolet light C.

have also been studied for their anticancer and chemopreventative effects in a variety of cancers including: skin cancer, lung cancer, colon cancer, and breast cancer (28–31). Aspirin is a potent inhibitor of COX activities and, therefore, prevents the synthesis of prostaglandins (27, 32–34). This has been the mechanism of action used to account for the activity of aspirin, but some recent studies have pointed toward other possible mechanisms. One such study looked at TPA tumor promotion activity in COX-1 and COX-2 knockout mice. This study showed that the knockout mice exhibited the same TPA-induced ear swelling as the wild-type mice (35–37). Aspirin inhibits COX activity through irreversible acetylation; however, not all of the NSAIDs have the ability to acetylate COX but are still active agents in reducing inflammation and/or cancer rates. NSAIDs have been shown to inhibit nuclear factor κ B as well as CAAT/enhancer binding protein β (38). Recently, aspirin and NAS, another NSAID, has been shown to inhibit UVB-induced AP-1 activation, both *in vivo* (39) and *in vitro* (40).

Here we demonstrate the *in vivo* chemopreventive effects of topically applied aspirin and NAS on UVB-induced non-melanoma skin cancer in the SKH-1 hairless mouse model. We then investigated potential mechanisms by which these agents inhibit UVB-induced AP-1 activation including inhibition of UVB-induced thymine dimer formation in the mouse skin epidermis.

Materials and Methods

Chemicals, Antibodies, Reagents, and Equipment. NAS was purchased from Fisher Scientific (Fair Lawn, NJ). Acetylsalicylic acid (aspirin), DMSO, and diaminobenzidine reagent were purchased from Sigma Chemical Co. (St. Louis, MO). Acetone was purchased from Mallinckrodt Baker, Inc. (Paris, KY). OMC was purchased from Acros Organics (Morris Plains, NJ). Vanicream was purchased from Pharmaceutical Specialties, Inc. (Rochester, MN). DMEM was purchased from Life Technologies, Inc. (Grand Island, NY). Monoclonal antibody to cyclobutane thymine dimers (clone KTM53, IgG1) was purchased from Kamiya Biomedical Company (Seattle, WA). Biotin-conjugated goat antimouse IgG1 was part of a MOM kit purchased from Vector Laboratories (Burlingame, CA). Westinghouse FS-40 UVB lamps were purchased from National Biological Corp. (Twinsburg, OH), and a UVX digital radiometer with a UVX-31 sensor was purchased from UV Products Inc. (San Gabriel, CA). Approximately 80% of the lamp output was in the UVB (290–320 nm), <1% in the UVC (<290 nm), 4% in the UVA (320–400 nm), and the remainder was in the visible spectrum according to manufacturer's specifications.

Mouse. Six-week-old pathogen-free female SKH-1 mice were purchased from Charles River Laboratory (Wilmington, MA). Animals were maintained in microisolators and cared for according to the United States Department of Health and Human Services Guidelines for Animal Care.

Chemoprevention of Carcinogenesis Protocol. Mice were randomly divided into treatment groups of 20. One week before the start of UVB irradiation and throughout the duration of the experiment, the mice were treated topically three times weekly with aspirin (10 or 40 μ mol), NAS (10 or 40 μ mol), or vehicle control. One hundred μ l of aspirin, in acetone, and 75 μ l NAS, in Vanicream, were applied to the dorsal surface 1 h before UVB irradiation. Aspirin was insoluble in Vanicream, and NAS was insoluble in acetone; therefore, two different vehicle controls were used. Animals were irradiated three times a week initially with a 1.5 kJ/m² dose and escalating weekly by 1.5 kJ/m² to a final dose of 9.0 kJ/m². UVB flux of the lamps was

measured weekly with a cosine-correcting UV digital radiometer described above. Cages were systematically rotated to compensate for differences in flux at the various positions. Mice were examined weekly for palpable tumors, which after onset were measured once a week. The mice were also weighed weekly, and at the end of the experiment tumors were taken for pathological analysis. The majority of the tumors at the end of the experiment were determined to be squamous cell carcinomas.

In Vivo Luciferase Assays. Previously generated AP-1-luciferase transgenic mice (B6D2) expressing a luciferase reporter driven by a heterologous promoter containing four TPA-response elements were punched biopsied (1.5 mm) on the dorsal skin to determine the basal level of luciferase activity (26). Two weeks later, the mice were irradiated with 10 kJ/m² of UVB, and another punch biopsy was taken to determine the UVB induction of AP-1 transcriptional activity. After another 2-week recovery period, the mice were treated twice over 3 days with either 75 μ l/mouse hydrophilic cream, 40 μ mol/mouse NAS in hydrophilic cream, or 40 μ mol/mouse 2-ethylhexyl salicylate in hydrophilic cream. 2-Ethylhexyl salicylate is insoluble in Vanicream, so hydrophilic cream was used. Three h after the last drug treatment, the mice were irradiated with 10 kJ/m² of UVB. Punched biopsies were taken 48 h later to determine the effect of NAS and 2-ethylhexyl salicylate on UVB induction of AP-1 activity in the epidermis. Luciferase activity of punch biopsied epidermis was measured as described previously (26).

Preparation of Thymine Dimer Skin Samples. Mice were divided randomly into treatment groups of 3. They were topically treated with either NAS (10 or 40 μ mol) in Vanicream, aspirin (40 μ mol) in acetone, or OMC (10 μ mol) in DMSO. One hundred μ l of aspirin, 75 μ l NAS, and 100 μ l of OMC were applied to the dorsal surface 1 h before 2 kJ/m² UVB irradiation. Immediately after UVB irradiation, the mice were sacrificed, and 4-mm punch biopsies of the treated dorsal skin were taken and placed in 10% phosphate-buffered formalin at 4°C for 24 h. The skin samples were processed and paraffin-embedded. Five- μ m sections of skin containing epidermis and dermis were made, deparaffinized, rehydrated with water, and used for immunohistochemical staining.

Immunostaining of Thymine Dimers. Thymine dimers in epidermal cells were detected using a monoclonal antibody to thymine dimers. The immunohistochemistry procedure has been described previously (41); however, a brief description follows. Slides were deparaffinized and incubated for 10 min at 95°C in Dako Target antigen retrieval solution (Dako Corp., Carpinteria, CA). The slides were then rinsed in water, incubated in 3% H₂O₂ for 5 min, then blocked with avidin and biotin (Vector Laboratories). Using Vector's MOM kit, the slides were blocked for 1 h with an IgG blocking reagent, rinsed, incubated for 5 min in the MOM kit working solution, and then incubated with the primary antibody at 1:500 for 1 h. After a brief wash in PBS, secondary antimouse biotin (Vector MOM) was then applied for 10 min. This was followed by a ABC peroxidase kit (Vector) for 5 min. The slides were developed with 3,3'-diaminobenzidine for 6 min. The sections were then rinsed and counterstained with a 1:4 dilution of Harris hematoxylin, cleared, and mounted. The 3,3'-diaminobenzidine-peroxidase reaction gave a dark brown reaction product, whereas the hematoxylin gave a light blue nuclear counterstain.

Analysis of Thymine Dimer-positive Cells. To determine the inhibitory effect of NAS, aspirin, and OMC on UVB-induced thymine dimer formation in the mouse epidermis, the stained

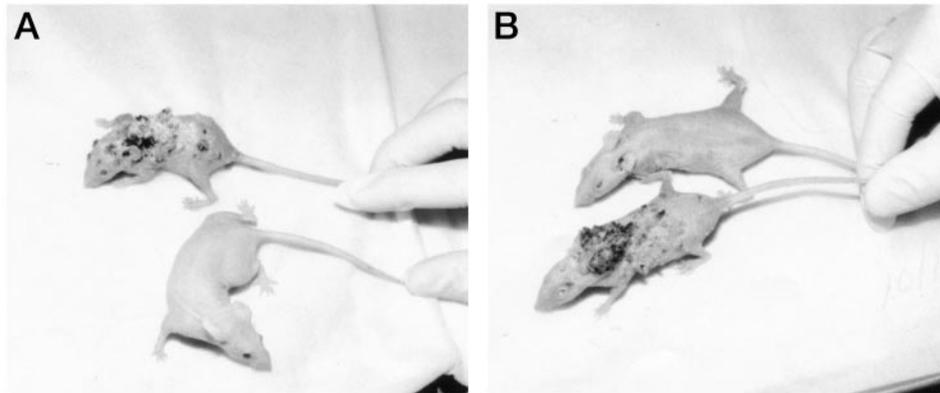


Fig. 1. Effect of NAS on UVB-induced skin damage. Groups of 20 female SKH-1 mice were treated with Vanicream or 40 μmol NAS (in Vanicream) applied topically to the dorsal surface. Vehicle control mice received comparable treatment. Applications were given three times weekly. One week after the start of NAS treatment, UVB treatments began. Mice were irradiated 3 days a week with 9.0 kJ/m^2 UVB. Photographs were taken after 23 weeks. **A**, the effect of UVB irradiation on mouse skin. Unirradiated mouse (*bottom*) and UVB-irradiated mouse (*top*). **B**, the effect of topical application of 40 μmol NAS on UVB-induced mouse skin irritation. Forty μmol NAS + UVB-treated mouse (*top*) and Vanicream + UVB-treated mouse (*bottom*).

tissue was counted at four to five random locations using an ocular micrometer grid under $\times 400$ magnification. After counting each individual tissue sample, the number of thymine dimer-positive cells in the epidermis was expressed as a percentage \pm SD of the mean from all 3 of the treated mice per group. Images from the immunostaining experiments were obtained using a Leica DMR microscope. The images were scanned using image quant software and were formatted as tiff images in Adobe Photoshop.

Statistical Method. Statistical analysis was done using Analysis of Variance model (42) and the Generalized Estimating Equation model (43). For the average tumor area and tumor burden, the growth curve for each mouse was estimated by least squares regression lines of logarithm of average tumor area and tumor burden by week (from week 14 to week 25). The logarithm was used because it linearized the growth curve. The slope of the regression line is interpreted as the average tumor area growth rate or tumor burden growth rate. The one-way Analysis of Variance was used to test for the NAS or aspirin effect on the tumor growth rate. For the difference in tumor incidence and tumor multiplicity between NAS and Vanicream, between aspirin and acetone, the Generalized Estimating Equation model was used. For UVB-induced thymine dimer formation, one-way Analysis of Variance was used to test the difference between Vanicream and the NAS/aspirin-treated groups. All of the statistical tests used an α of 0.05 significance level.

Results

Inhibition of UVB Photocarcinogenesis by NAS and Aspirin. Mice were treated topically with 10 or 40 μmol NAS or aspirin three times weekly throughout the experiment. Treatments began 1 week before UV irradiation, and after the onset of UV treatment, NAS and aspirin were applied 1 h before UV. Mice were irradiated 3 days a week at a final dose of 9.0 kJ/M^2 . This regimen of UVB produced both squamous cell carcinomas ($>90\%$) and papillomas ($<10\%$; data not shown). It has been shown that chronic UV irradiation causes a reduced body weight relative to unirradiated controls. Topical application of both NAS and aspirin did not cause any change in body weight or behavior in irradiated mice (data not shown) suggesting no systemic toxicity.

After ~ 3 weeks of irradiation, the dorsal skin became dry and chapped, and peeling was observed. As shown in Fig. 1B, repeated topical NAS applications had a visible, protective effect against UV-induced skin irritation. Tumors began to appear consistently starting at week 14. Tumor formation was visible first as localized swelling, followed by papilloma-like peaks that grew to measurable tumors. Most ($>94\%$) of the counted tumors were localized to the dorsal surface with the remaining tumors formed on the head. There were a few ocular tumors that were not included in the data. UVB treatment was discontinued after 10 weeks, when the tumor incidence exceeded 90%. Tumor regression was rare ($<3\%$). The results presented are representative of two separate experiments.

Fig. 2, A and B, show that 10 and 40 μmol of NAS delayed the appearance of tumors. Percentage of incidence represents the proportion of mice bearing ≥ 1 tumors. Multiplicity represents the average number of tumors/mouse. At week 22 of irradiation, control mice had an average of ~ 4.08 tumors/mouse and an incidence of 92%. At week 22, 40 μmol of NAS-treated mice had only 1.08 tumors/mouse with an incidence of 58%; in comparison, the 40 μmol aspirin-treated mice had ~ 2.5 tumors/mouse and 100% incidence. The incidence and multiplicity of tumors increased slowly in the 10 and 40 μmol groups, but the values remained significantly below that of the control through week 23. As compared with Vanicream controls, 10 μmol of NAS showed a statistically significant inhibition of the rate of incidence ($P = 0.0006$) and multiplicity ($P = 0.0003$). The 40 μmol NAS also showed a statistically significant inhibition of both rate of tumor incidence ($P = 0.0003$) and the rate of tumor multiplicity ($P = 0.0001$). Forty μmol of NAS were not significantly more effective than 10 μmol at inhibiting the rate of tumor incidence ($P = 0.6744$) but were significantly more effective than 10 μmol of NAS in inhibiting the rate of tumor multiplicity ($P = 0.032$). Combined treatment of Vanicream and UVB was significantly better at increasing the rate of tumor incidence ($P = 0.0038$) and the rate of tumor multiplicity ($P = 0.0015$).

Average tumor burden was measured as the average total area of all of the tumors on a single mouse (Fig. 3A). Average tumor areas (Fig. 3B) indicate the average size of an individual tumor, a calculation of length \times height as measured by calipers.

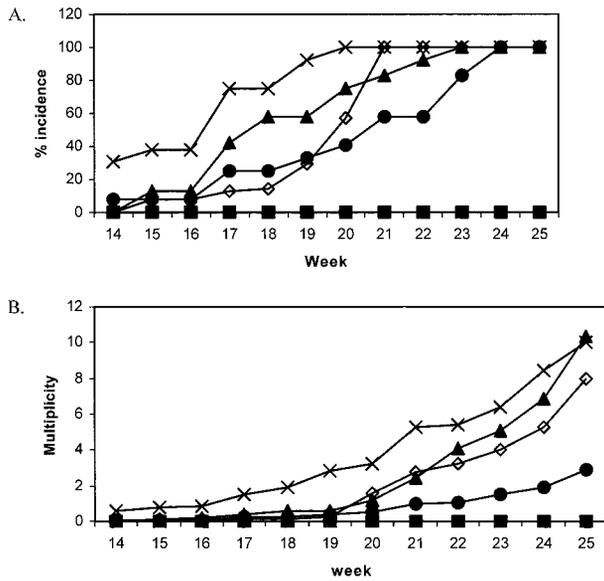


Fig. 2. Tumor formation in UVB-irradiated mice treated with NAS. Groups of 20 female SKH-1 mice were treated with 10 or 40 μmol NAS in Vanicream applied to the dorsal surface. Controls received the same treatment, except Vanicream. Topical applications were given three times weekly. One week after the start of NAS treatment, UVB treatments began. Mice were irradiated 3 days a week with 9.0 kJ/m^2 UVB. Irradiation and NAS treatment were discontinued after week 25. ■, -UVB; ▲, +UVB; ×, Vanicream, +UVB; □, 10 μmol NAS, +UVB; and ●, 40 μmol NAS, +UVB. **A**, effect of NAS on UVB-induced tumor incidence. Incidence was expressed as a percentage of the number of tumor-bearing mice divided by the number of alive mice. Both 10 and 40 μmol NAS significantly inhibited the rate of incidence, $P = 0.0006$ and $P = 0.0003$, respectively. **B**, effect of NAS on UVB-induced tumor multiplicity. Multiplicity was expressed as the mean of the number of tumors/mouse. Both 10 and 40 μmol NAS significantly inhibited the rate of tumor multiplicity, $P = 0.0003$ and $P = 0.0001$, respectively.

Both 40 μmol of NAS ($P = 0.0019$) and 10 μmol of NAS ($P = 0.0001$) significantly lowered the average rate of tumor burden per mouse when compared with the Vanicream control mice. Forty μmol of NAS was not more effective in lowering the average rate of tumor burden per mouse than 10 μmol of NAS ($P = 0.1966$). When compared with the Vanicream control, 10 μmol of NAS significantly inhibited the rate of average tumor area growth (Fig. 3B; $P = 0.0004$), whereas the 40 μmol dose did not ($P = 0.1769$). Combined treatment of Vanicream and UVB did not significantly alter the average rate of tumor burden ($P = 0.0775$) or the average rate of tumor area ($P = 0.1608$) when compared with the UVB alone treated group.

As shown in Fig. 4A, both 10 μmol and 40 μmol of aspirin did significantly delay the rate of tumor incidence ($P = 0.0093$ and $P = 0.0285$, respectively). As compared with acetone controls, 10 μmol of aspirin did not significantly inhibit tumor multiplicity ($P = 0.0933$; Fig. 4B). However, the 40 μmol of aspirin did significantly inhibit tumor multiplicity ($P = 0.0030$) when compared with the acetone control. Forty μmol of aspirin were not more effective than 10 μmol of aspirin in lowering the rate of tumor incidence ($P = 0.7173$); however, 40 μmol of aspirin were more effective than 10 μmol in lowering the rate of tumor multiplicity ($P = 0.0153$). Combined treatment of acetone and UVB was significantly better at increasing the rate of tumor incidence ($P = 0.0010$) and the rate of tumor multiplicity ($P = 0.0103$). Neither dose of aspirin significantly inhibited the average rate of tumor burden ($P = 0.5559$ for 10 μmol and $P = 0.7478$ for 40 μmol) or the average rate of tumor

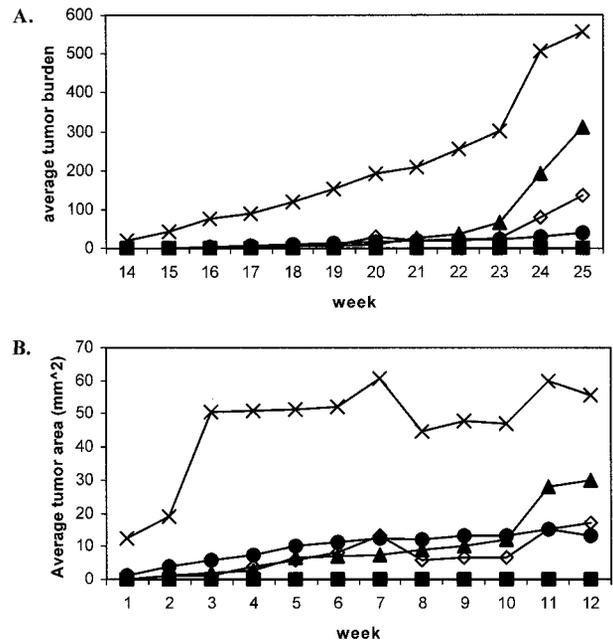


Fig. 3. Tumor growth in UVB-irradiated mice treated with NAS. Groups of 20 female SKH-1 mice were treated with 10 or 40 μmol NAS in Vanicream applied to the dorsal surface. Controls received the same treatment, except Vanicream. Topical applications were given three times weekly. One week after the start of NAS treatment, UVB treatments began. Mice were irradiated 3 days a week with 9.0 kJ/m^2 UVB. Irradiation and NAS treatment were discontinued after week 25. ■, -UVB; ▲, +UVB; ×, Vanicream, +UVB; □, 10 μmol NAS, +UVB; and ●, 40 μmol NAS, +UVB. **A**, effects of NAS on UVB-induced tumor area per mouse. Tumor burden was expressed as the mean total area of all tumors on a mouse. Both 10 and 40 μmol NAS significantly inhibited the rate of tumor burden per mouse, $P = 0.0001$ and $P = 0.0019$, respectively. **B**, effects of NAS on UVB-induced tumor size. Tumor area was expressed as the mean of the size of each individual tumor. Ten μmol NAS significantly lowered the rate of the average tumor area, $P = 0.0004$. Forty μmol NAS did not significantly lower the rate of the average tumor area, $P = 0.1769$.

area ($P = 0.7815$ for 10 μmol and $P = 0.7103$ for 40 μmol). Combined treatment of acetone and UVB was not significantly better at increasing the average rate of tumor burden ($P = 0.9485$) or the average rate of tumor area ($P = 0.9081$; Fig. 5, A and B).

Tumor formation was inhibited by 10 and 40 μmol of NAS and 40 μmol of aspirin, but was not completely prevented. At all of the later time points shown, the 10 and 40 μmol of NAS and 40 μmol of aspirin treated groups had a significantly lower proportion of tumor-bearing mice. The positive UVB control showed a dramatic increase in tumor multiplicity starting at week 21 and a dramatic increase in percentage of incidence at week 16. Forty μmol of NAS delayed tumor formation by ~4 weeks. The 10 μmol of NAS, as well as the 40 μmol of aspirin also delayed tumor formation, but not as effectively as 40 μmol of NAS.

In Vivo Inhibition of AP-1 Transactivation by NAS and 2-Ethylhexyl Salicylate. A 4X-TPA-response element-luciferase element, shown to be transactivated by AP-1, transgenic mouse model was used to measure *in vivo* AP-1 transactivation. Ten kJ/m^2 UVB induced an ~22-fold increase in luciferase activity over basal levels, whereas UVB plus hydrophilic cream produced an ~29-fold increase (Fig. 6). In the same mice, 40 μmol of NAS produced an ~36% inhibition of luciferase activity when compared with the hydrophilic cream UVB ve-

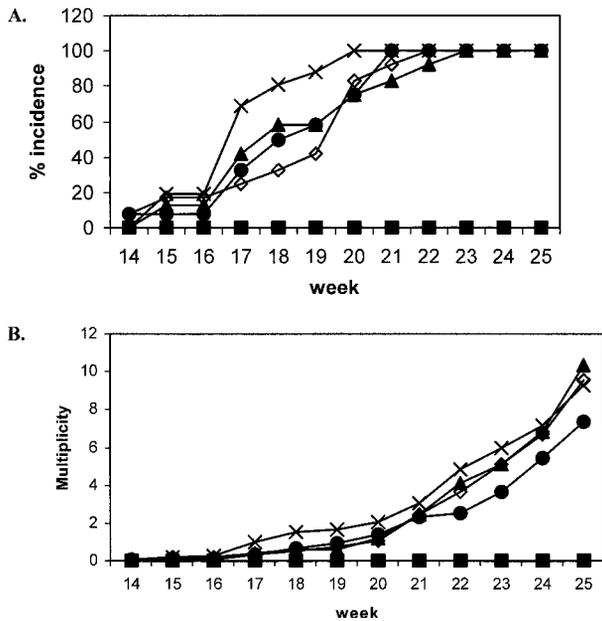


Fig. 4. Tumor formation in UVB-irradiated mice treated with aspirin. Groups of 20 female SKH-1 mice were treated with 10 or 40 μmol aspirin in acetone applied to the dorsal surface. Controls received the same treatment, except acetone. Topical applications were given three times weekly. One week after the start of aspirin treatment, UVB treatments began. Mice were irradiated 3 days a week with 9.0 kJ/m^2 UVB. Irradiation and NAS treatment was discontinued after week 25. ■, -UVB; ▲, +UVB; ×, Acetone, +UVB; □, 10 μM ASP, +UVB; and ●, 40 μM ASP, +UVB. **A**, effects of aspirin on UVB-induced tumor incidence. Incidence was expressed as a percentage of the number of tumor-bearing mice divided by the number of alive mice. Both 10 and 40 μmol of aspirin significantly inhibited the rate of incidence, $P = 0.0093$ and $P = 0.0285$, respectively. **B**, effects of aspirin on UVB-induced tumor multiplicity. Multiplicity was expressed as the mean of the number of tumors per mouse. Forty μmol of aspirin significantly inhibited the rate of tumor multiplicity, $P = 0.0030$. Ten μmol NAS did not significantly lower the rate of tumor multiplicity.

hicle control. Ten μmol of 2-ethylhexyl salicylate, a commercial sun-screening agent, produced a 21% inhibition of luciferase activity when compared with the hydrophilic cream UVB control. The inhibition produced by NAS and 2-ethylhexyl salicylate was found to be significantly lower with $P = 0.0104$ and $P = 0.002$, respectively, as determined via a one-tailed distribution, two-sample unequal variance.

Inhibition of UVB-induced Thymine Dimer Formation by NAS and OMC. Thymine dimers are found in DNA immediately on absorption of UVB and, therefore, can be used as a biomarker to study sun-screening agents if dimers are measured immediately after irradiation. As shown in Fig. 7B, exposure of mouse skin to 2 kJ/m^2 UVB induces thymine dimer formation in the DNA of epidermal cells. Two kJ/m^2 UVB was used in these experiments because 9 kJ/m^2 UVB, the final dose used in the carcinogenesis experiments above, produced too many thymine dimers making detection of the reduction in thymine dimer formation more difficult. Thymine dimers were not detected in non-UVB irradiated mouse epidermis (Fig. 7A). Treatment with 40 μmol of aspirin did not inhibit UVB-induced thymine dimer formation in the mouse epidermis ($P = 0.9999$; Fig. 7C), whereas treatment with 10 μmol of OMC, an agent used in commercial sunscreens, did inhibit UVB-induced dimer formation ($P = 0.0001$; Fig. 7D). OMC is a more potent sunscreen agent than 2-ethylhexyl salicylate, absorbing more in the UVB wavelength; therefore, it was a better candidate for

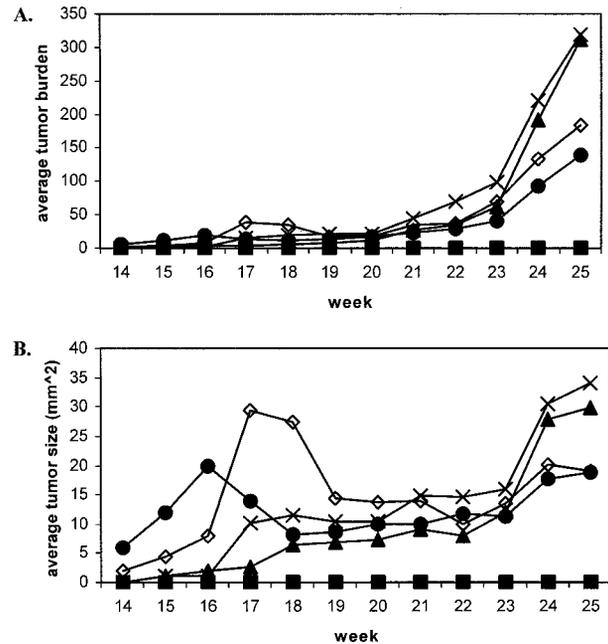


Fig. 5. Tumor growth in UVB-irradiated mice treated with aspirin. Groups of 20 female SKH-1 mice were treated with 10 or 40 μmol aspirin in acetone applied to the dorsal surface. Controls received the same treatment, except acetone. Topical applications were given three times weekly. One week after the start of aspirin treatment, UVB treatments began. Mice were irradiated 3 days a week with 9.0 kJ/m^2 UVB. Irradiation and aspirin treatment was discontinued after week 25. ■, -UVB; ▲, +UVB; ×, Acetone, +UVB; □, 10 μM ASP, +UVB; and ●, 40 μM ASP, +UVB. **A**, effects of aspirin on UVB-induced tumor area per mouse. Tumor burden was expressed as the mean total area of all tumors on a mouse. Ten and 40 μmol aspirin did not significantly inhibit the rate of tumor burden per mouse, $P = 0.5559$ and $P = 0.7478$, respectively. **B**, effects of aspirin on UVB-induced tumor size. Tumor area was expressed as the mean of the size of each individual tumor. Ten and 40 μmol aspirin did not significantly inhibit the average rate of tumor area, $P = 0.7815$ and $P = 0.7103$, respectively.

these studies (44). Ten μmol of NAS did not significantly inhibit UVB-induced dimer formation in the mouse epidermis ($P = 0.0697$), whereas 40 μmol of NAS did significantly inhibit UVB-induced dimer formation ($P = 0.0001$; Fig. 7, E and F). The Vanicream vehicle control had no effect on UVB-induced dimer formation ($P = 0.9996$). Quantitative analysis of UVB-treated mouse epidermis showed that $85.91\% \pm 2.70$ of the nuclei staining positive for dimer formation. Irradiated mice treated with 40 μmol of aspirin had $86.26\% \pm 3.57$ epidermal nuclei positive for dimer formation, whereas irradiated mice treated with 10 and 40 μmol of NAS showed $60.36\% \pm 9.08$ and $29.52\% \pm 25.19$ epidermal dimer formation, respectively. Treatment of irradiated mice with 40 μmol of OMC showed a $34.97\% \pm 8.61$ epidermal dimer formation (Fig. 7F).

Discussion

In this study, we have shown for the first time that topical application of NAS and aspirin can inhibit UVB-induced non-melanoma skin cancer. In the SKH-1 mouse model, topical application of NAS inhibited tumor incidence, multiplicity, and average tumor burden/mouse. NAS delayed the carcinogenic effect of UVB without any observable toxicity. UVB-induced skin damage also appeared to be prevented by topical treatment of NAS. We found that NAS can prevent the formation of UVB-induced thymine dimer in the mouse epidermis, suggesting a sun-screening mechanism of action. Topical application

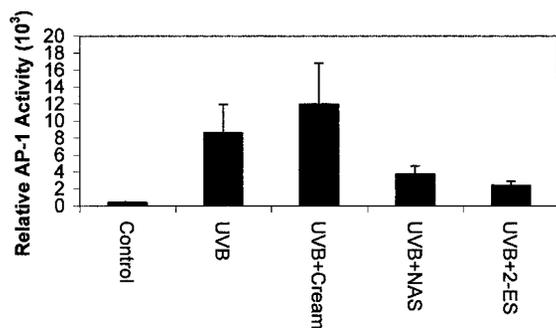


Fig. 6. Effect of NAS and 2-ethylhexyl salicylate on UVB-induced AP-1 transactivation *in vivo*. AP-1-luciferase transgenic mice were punch biopsied for basal activity and at 48 h after a single dose of 10 kJ/m² UVB. Two weeks later, mice received two treatments over 3 days of 40 μ mol NAS, 40 μ mol 2-ethylhexyl salicylate, or 75 μ l hydrophilic cream carrier before a single of 10 kJ/m² UVB. Forty-eight h later, mice were punch biopsied. Luciferase assay was carried out to determine AP-1 transactivation. Both NAS and 2-ES significantly inhibited UVB-induced AP-1 activation, $P = 0.0104$ and $P = 0.002$, respectively. The data in this figure represent the mean of luciferase values from 16 mice; bars, \pm SD.

of aspirin, in the SKH-1 mouse model, inhibited tumor incidence and multiplicity but did not inhibit the average tumor burden. These results indicate that topical aspirin can protect against tumor formation while not effecting tumor growth. Aspirin showed no protective effect against UVB-induced skin damage and also failed to inhibit thymine dimer formation in the mouse epidermis, indicating that aspirin does not act as a sunscreen.

UVB photocarcinogenesis of the skin is a multistep process involving initiation, promotion, and progression. Unlike some chemical carcinogens, UVB is a complete carcinogen, being able to play an important role in initiation, promotion, and progression (3). UVB acts as an initiator via generation of reactive oxygen species and direct DNA damage in the form of DNA photoproducts (4, 5). UVB-induced photoproducts can lead to mutations, and these dimers have been shown to be responsible for mutated p53 (11). Cells with mutated p53 are less prone to apoptosis and, therefore, may have a selective growth advantage during chronic UVB exposure. UVB promotion occurs by the activation of certain oncogenic transcription factors such as AP-1 and oncogenic genes such as COX-2 (45, 46).

UVB-induced nonmelanoma skin cancer can be prevented at either the initiation stage and/or the promotion/progression stage. Sunscreen agents act to prevent UVB initiation by absorbing UVB before it interacts with the cell, thus preventing UVB carcinogenesis. OMC and 2-ethylhexyl salicylate, commercially used sunscreen agents, have been described as strongly absorbing in the UVB wavelength (280–320 nm), and, therefore, act as good sun-screening agents (44). Thymine dimers form immediately on DNA absorption of UVB energy, and, therefore, represent an early biomarker of UVB initiation. Treatment with OMC before UVB exposure greatly reduced thymine dimer formation demonstrating the protective effects of sunscreen on UVB initiation. We found NAS to also absorb in the UVB wavelength (data not shown). Treatment before UVB exposure also inhibited UVB-induced thymine dimer formation in the same manner as OMC.

AP-1 has been shown to be up-regulated and activated by UVB. Being a potent oncogene transcription factor, AP-1 is an end point biomarker that allows one to observe the promotional activity of UVB. The absorption of UVB by sunscreen not only prevents UVB initiation, but also promotion. We have demon-

strated this by showing that 2-ethylhexyl salicylate, a known sunscreen agent, can inhibit UVB-induced AP-1 *in vivo*, when applied before UVB exposure. UVB irradiation has been shown previously to induce tumors in the B6D2 mouse strain used for these studies, and, therefore, inhibition of AP-1 is because of drug treatment and not mouse strain differences (47). When applied before UVB exposure, NAS exhibited a similar degree of inhibition of UVB-induced AP-1 *in vivo* as did 2-ethylhexyl salicylate.

Treatment with NAS before UVB exposure inhibited UVB-induced AP-1 activation *in vivo* similar to the inhibition seen with 2-ethylhexyl salicylate. These results, along with the thymine dimer results, indicate that NAS acts in the same manner as known sunscreen agents, and, therefore, inhibits UVB-induced carcinogenesis by a sunscreen mechanism.

It is important to note that NAS did not completely inhibit tumor formation in the UVB carcinogenesis experiment nor did it completely inhibit UVB-induced thymine dimer formation in the epidermis of the mice. This observation can be explained by the manner in which NAS was applied to the animals. It was applied in Vanicream, which was rubbed onto the backs of the mice. It is possible that the spreading of the cream across the back was not even, therefore, resulting in a nonuniform application of NAS. In addition, the mice were treated 1 h before irradiation, allowing time for the mice to lick or scratch at their treated backs. Vanicream does not evaporate off the back of the mouse nor absorb as fast as acetone, thus, over the course of an hour of licking and scratching, some of the drug could be removed from part of the mouse back, decreasing the protective effect of NAS. Thus, a nonuniform pattern of protection along the backs of the mice may have resulted.

Aspirin has been shown previously to not absorb in the UVB part of the light spectrum (39), and treatment with aspirin before UVB did not inhibit thymine dimer formation in the mouse epidermis. These results indicate that aspirin is not a sunscreen. However, aspirin did protect against UVB-induced tumor formation in the SKH-1 mouse model by significantly inhibiting the rate of tumor incidence and the rate of tumor multiplicity. Aspirin has been shown to both inhibit AP-1 and COX-2 activities, and this may explain the protective effect seen here. A pretreatment with 2 mM of aspirin inhibited AP-1 activity in the JB6 mouse keratinocytes cell line (39). It was also found that this dose of aspirin inhibited the UVB activation of the mitogen-activated protein kinases: extracellular signal-regulated kinase, p38, and c-Jun NH₂-terminal kinase (39). Because these MAPKs have been shown to activate AP-1, inhibition of these kinases by aspirin would explain the observed inhibition of AP-1. Aspirin has been shown recently to inhibit IL-1 β , and phorbol 12-myristate 13-acetate induced COX-2 mRNA induction and COX-2 promoter activity (48). Therefore, the reduction in tumor formation seen with 40 μ mol of aspirin treatment may be because of inhibition of UVB-induced AP-1 and/or COX-2 activation *in vivo*.

NAS has also been shown to prevent phorbol 12-myristate 13-acetate, and IL-1 β induced COX-2 transactivation and mRNA induction (48). This inhibition may be achieved by preventing CAAT/enhancer binding protein β binding to the COX-2 promoter (38). UVB was not used in these studies, and, therefore, this inhibition is through a nonsunscreen mechanism. It has been shown that UVB irradiation of mouse skin can increase IL-1 β activation (49). These results suggest that NAS may prevent UVB carcinogenesis by both a sunscreen mechanism and a nonsunscreen mechanism.

Because it is not acting as a sunscreen, the topical treatment of aspirin used in this study may not have been used at a

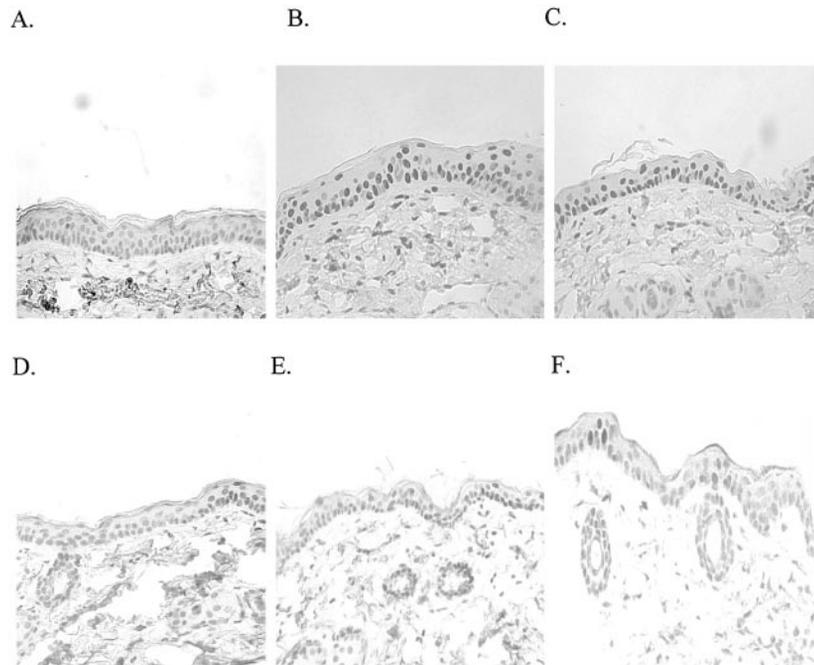
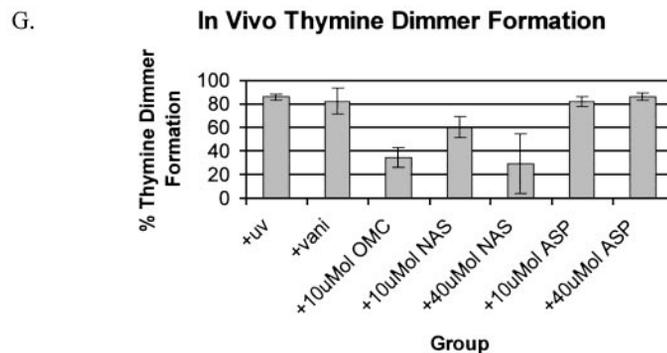


Fig. 7. Effects of sodium salicylate, aspirin, and octyl-4-methoxycinnamate on UVB-induced thymine dimer formation in mouse skin. Immunostaining was performed to detect UVB-induced DNA photodamage in the form of thymine dimer-positive cells, stained dark brown. Thymine dimers were not detected in non-UVB-exposed cells (A). Two kJ/m^2 UVB induced thymine dimer formation in mouse epidermal nuclei (B) immediately after UVB exposure. Topical treatment with 40 μmol aspirin before UVB exposure had no protective effect on dimer formation (C). A pretreatment with 10 μmol OMC significantly decreased ($P = 0.0001$) UVB-induced thymine dimer formation (D). This protective effect was also seen with a pretreatment of 40 μmol NAS, $P = 0.0001$ (F). There was not a significant inhibition of UVB-induced thymine dimer formation with a pretreatment of 10 μmol NAS treatment, $P = 0.0697$ (E). These slides are representative of 3 different mice. The number of thymine dimer-positive cells in the epidermis was counted under the microscope using an ocular grid as described in "Materials and Methods." The number of thymine dimer-positive cells are represented as the mean percentage of thymine dimer-positive cells immediately after UVB exposure (G); bars, \pm SD.



high enough concentration to prevent UVB-induced tumor growth. Although a 40- μmol dose of aspirin has been shown to inhibit AP-1 *in vivo* after a single dose of UVB, it may not be enough to completely inhibit chronic UVB-induced AP-1. In addition, UVB can activate many other oncogenes, some of which may not be inhibited by aspirin. The dose-dependent results seen with NAS suggest that higher doses of aspirin may be more effective.

As a sunscreen agent, NAS inhibits UVB-induced initiation and promotion/progression; however, in contrast to other sunscreen agents, NAS also can inhibit the promotion stage of skin carcinogenesis at the level of preventing UVB-mediated signal transduction leading to AP-1 activation. The ability to act as a sunblock or screen and, at the same time being able to prevent UVB-induced signal transduction may make NAS a more potent chemoprevention agent than some of the commercial sunscreens used now.

References

1. Koh, H. K. Preventive strategies and research for ultraviolet-associated cancer. *Environ. Health Perspect.*, 103(Suppl. 8): 255–257, 1995.

- Learn, D. B., Beasley, D. G., Giddens, L. D., Beard, J., Stanfield, J. W., and Roberts, L. K. Minimum doses of ultraviolet radiation required to induce murine skin edema and immunosuppression are different and depend on the ultraviolet emission spectrum of the source. *Photochem. Photobiol.*, 62: 1066–1075, 1995.
- Ananthaswamy, H. N., and Pierceall, W. E. Molecular mechanisms of ultraviolet radiation carcinogenesis. *Photochem. Photobiol.*, 52: 1119–1136, 1990.
- Ghosh, R., Amstad, P., and Cerutti, P. UVB-induced DNA breaks interfere with transcriptional induction of c-fos. *Mol. Cell. Biol.*, 13: 6992–6999, 1993.
- Lehman, T. A., Modali, R., Boukamp, P., Stanek, J., Bennett, W. P., Welsh, J. A., Metcalf, R. A., Stampfer, M. R., Fusenig, N., Rogan, E. M., and *et al.* p53 mutations in human immortalized epithelial cell lines. *Carcinogenesis (Lond.)*, 14: 833–839, 1993.
- Ronai, Z. A., Okin, E., and Weinstein, I. B. Ultraviolet light induces the expression of oncogenes in rat fibroblast and human keratinocyte cells. *Oncogene*, 2: 201–204, 1988.
- Schwarz, T., and Luger, T. A. Effect of UV irradiation on epidermal cell cytokine production. *J. Photochem. Photobiol. B*, 4: 1–13, 1989.
- Rosette, C., and Karin, M. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science (Wash. DC)*, 274: 1194–1197, 1996.
- Garmyn, M., Yaar, M., Boileau, N., Backendorf, C., and Gilchrist, B. A. Effect of aging and habitual sun exposure on the genetic response of cultured human keratinocytes to solar-simulated irradiation. *J. Investig. Dermatol.*, 99: 743–748, 1992.

10. Schwarz, A., Bhardwaj, R., Aragane, Y., Mahnke, K., Riemann, H., Metzger, D., Luger, T. A., and Schwarz, T. Ultraviolet-B-induced apoptosis of keratinocytes: evidence for partial involvement of tumor necrosis factor- α in the formation of sunburn cells. *J. Invest. Dermatol.*, 104: 922–927, 1995.
11. Ziegler, A., Jonason, A. S., Leffell, D. J., Simon, J. A., Sharma, H. W., Kimmelman, J., Remington, L., Jacks, T., and Brash, D. E. Sunburn and p53 in the onset of skin cancer. *Nature (Lond.)*, 372: 773–776, 1994.
12. Gensler, H. L. Enhancement of chemical carcinogenesis in mice by systemic effects of ultraviolet irradiation. *Cancer Res.*, 48: 620–623, 1988.
13. Gensler, H. L., Simpson, P. J., and Powell, M. B. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion in murine skin by systemic effects of ultraviolet irradiation. *Photochem. Photobiol.*, 56: 25–30, 1992.
14. Husain, Z., Yang, Q. M., and Biswas, D. K. cHa-ras proto-oncogene. Amplification and overexpression in UV-B- induced mouse skin papillomas and carcinomas. *Arch. Dermatol.*, 126: 324–330, 1990.
15. Hart, R. W., Setlow, R. B., and Woodhead, A. D. Evidence that pyrimidine dimers in DNA can give rise to tumors. *Proc. Natl. Acad. Sci. USA*, 74: 5574–5578, 1977.
16. Sachsenmaier, C., Radler-Pohl, A., Zinck, R., Nordheim, A., Herrlich, P., and Rahmsdorf, H. J. Involvement of growth factor receptors in the mammalian UV-C response. *Cell*, 78: 963–972, 1994.
17. Huang, C., Ma, W., Ding, M., Bowden, G. T., and Dong, Z. Direct evidence for an important role of sphingomyelinase in ultraviolet-induced activation of c-Jun N-terminal kinase. *J. Biol. Chem.*, 272: 27753–27757, 1997.
18. Huang, C., Ma, W., Bowden, G. T., and Dong, Z. Ultraviolet B-induced activated protein-1 activation does not require epidermal growth factor receptor but is blocked by a dominant negative PKC λ /iota. *J. Biol. Chem.*, 271: 31262–31268, 1996.
19. Adler, V., Pincus, M. R., Polotskaya, A., Montano, X., Friedman, F. K., and Ronai, Z. Activation of c-Jun-NH2-kinase by UV irradiation is dependent on p21ras. *J. Biol. Chem.*, 271: 23304–23309, 1996.
20. Young, M. R., Li, J. J., Rincon, M., Flavell, R. A., Sathyanarayana, B. K., Hunziker, R., and Colburn, N. Transgenic mice demonstrate AP-1 (activator protein-1) transactivation is required for tumor promotion. *Proc. Natl. Acad. Sci. USA*, 96: 9827–9832, 1999.
21. Domann, F. E., Levy, J. P., Birrer, M. J., and Bowden, G. T. Stable expression of a c-JUN deletion mutant in two malignant mouse epidermal cell lines blocks tumor formation in nude mice. *Cell Growth Differ.*, 5: 9–16, 1994.
22. Ostrowski, L. E., Finch, J., Krieg, P., Matrisian, L., Patskan, G., O'Connell, J. F., Phillips, J., Slaga, T. J., Breathnach, R., and Bowden, G. T. Expression pattern of a gene for a secreted metalloproteinase during late stages of tumor progression. *Mol. Carcinog.*, 1: 13–19, 1988.
23. Hashimoto, Y., Tajima, O., Hashiba, H., Nose, K., and Kuroki, T. Elevated expression of secondary, but not early, responding genes to phorbol ester tumor promoters in papillomas and carcinomas of mouse skin. *Mol. Carcinog.*, 3: 302–308, 1990.
24. Lamb, R. F., Hennigan, R. F., Turnbull, K., Katsanakis, K. D., MacKenzie, E. D., Birnie, G. D., and Ozanne, B. W. AP-1-mediated invasion requires increased expression of the hyaluronan receptor CD44. *Mol. Cell Biol.*, 17: 963–976, 1997.
25. Suzuki, T., Murakami, M., Onai, N., Fukuda, E., Hashimoto, Y., Sonobe, M. H., Kameda, T., Ichinose, M., Miki, K., and Iba, H. Analysis of AP-1 function in cellular transformation pathways. *J. Virol.*, 68: 3527–3535, 1994.
26. Huang, C., Ma, W. Y., Dawson, M. I., Rincon, M., Flavell, R. A., and Dong, Z. Blocking activator protein-1 activity, but not activating retinoic acid response element, is required for the antitumor promotion effect of retinoic acid. *Proc. Natl. Acad. Sci. USA*, 94: 5826–5830, 1997.
27. Meade, E. A., Smith, W. L., and DeWitt, D. L. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.*, 268: 6610–6614, 1993.
28. Rosenberg, L., Palmer, J. R., Zauber, A. G., Warshauer, M. E., Stolley, P. D., and Shapiro, S. A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. *J. Natl. Cancer Inst.*, 83: 355–358, 1991.
29. Schreinemachers, D. M., and Everson, R. B. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology*, 5: 138–146, 1994.
30. Peleg, II, Maibach, H. T., Brown, S. H., and Wilcox, C. M. Aspirin and nonsteroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer. *Arch. Intern. Med.*, 154: 394–399, 1994.
31. Thun, M. J., Namboodiri, M. M., and Heath, C. W., Jr. Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.*, 325: 1593–1596, 1991.
32. Brooks, P. M., and Day, R. O. Nonsteroidal antiinflammatory drugs—differences and similarities. *N. Engl. J. Med.*, 324: 1716–1725, 1991.
33. Mitchell, J. A., Akarasereenont, P., Thiemermann, C., Flower, R. J., and Vane, J. R. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc. Natl. Acad. Sci. USA*, 90: 11693–11697, 1993.
34. Vane, J. R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.*, 231: 232–235, 1971.
35. Langenbach, R., Morham, S. G., Tian, H. F., Loftin, C. D., Ghanayem, B. I., Chulada, P. C., Mahler, J. F., Lee, C. A., Goulding, E. H., Kluckman, K. D., and et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell*, 83: 483–492, 1995.
36. Morham, S. G., Langenbach, R., Loftin, C. D., Tian, H. F., Vouloumanos, N., Jennette, J. C., Mahler, J. F., Kluckman, K. D., Ledford, A., Lee, C. A., and et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell*, 83: 473–482, 1995.
37. DeWitt, D., and Smith, W. L. Yes, but do they still get headaches? *Cell*, 83: 345–348, 1995.
38. Saunders, M. A., Sansores-Garcia, L., Gilroy, D. W., and Wu, K. K. Selective Suppression of CCAAT/Enhancer-binding protein β binding and cyclooxygenase-2 promoter activity by sodium salicylate in quiescent human fibroblasts. *J. Biol. Chem.*, 276: 18897–18904, 2001.
39. Huang, C., Ma, W. Y., Hanenberger, D., Cleary, M. P., Bowden, G. T., and Dong, Z. Inhibition of ultraviolet B-induced activator protein-1 (AP-1) activity by aspirin in AP-1-luciferase transgenic mice. *J. Biol. Chem.*, 272: 26325–26331, 1997.
40. Dong, Z., Huang, C., Brown, R. E., and Ma, W. Y. Inhibition of activator protein 1 activity and neoplastic transformation by aspirin. *J. Biol. Chem.*, 272: 9962–9970, 1997.
41. Chadwick, C. A., Potten, C. S., Nikaido, O., Matsunaga, T., Proby, C., and Young, A. R. The detection of cyclobutane thymine dimers, (6–4) photolesions and the Dewar photoisomers in sections of UV-irradiated human skin using specific antibodies, and the demonstration of depth penetration effects. *J. Photochem. Photobiol. B*, 28: 163–170, 1995.
42. Pagano, M., and Gauvreau, K. Principles of Biostatistics, pp. 285–301. Belmont, CA: Wadsworth Publishing Company, 1993.
43. Jorgensen, B., and Tsao, M. Dispersion models and longitudinal data analysis. *Stat. Med.*, 18: 2257–2270, 1999.
44. McVean, M., and Liebler, D. C. Prevention of DNA photodamage by vitamin E compounds and sunscreens: roles of ultraviolet absorbance and cellular uptake. *Mol. Carcinog.*, 24: 169–176, 1999.
45. Chen, W., Tang, Q., Gonzales, M. S., and Bowden, G. T. Role of p38 MAP kinases and ERK in mediating ultraviolet-B induced cyclooxygenase-2 gene expression in human keratinocytes. *Oncogene*, 20: 3921–3926, 2001.
46. Chen, W., Borchers, A. H., Dong, Z., Powell, M. B., and Bowden, G. T. UVB irradiation-induced activator protein-1 activation correlates with increased c-fos gene expression in a human keratinocyte cell line. *J. Biol. Chem.*, 273: 32176–81, 1998.
47. Gensler, H. L. Prevention of chemically induced two-stage skin carcinogenesis in mice by systemic effects of ultraviolet irradiation. *Carcinogenesis (Lond.)*, 9: 767–769, 1988.
48. Xu, X. M., Sansores-Garcia, L., Chen, X. M., Matijevic-Aleksic, N., Du, M., and Wu, K. K. Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. *Proc. Natl. Acad. Sci. USA*, 96: 5292–5297, 1999.
49. Scordi, I. A., and Vincek, V. Timecourse study of UVB-induced cytokine induction in whole mouse skin. *Photodermatol. Photoimmunol. Photomed.*, 16: 67–73, 2000.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association
for Cancer Research

Inhibitory Effects of Sodium Salicylate and Acetylsalicylic Acid on UVB-induced Mouse Skin Carcinogenesis

Warner B. Bair III, Nancy Hart, Janine Einspahr, et al.

Cancer Epidemiol Biomarkers Prev 2002;11:1645-1652.

Updated version Access the most recent version of this article at:
<http://cebp.aacrjournals.org/content/11/12/1645>

Cited articles This article cites 47 articles, 19 of which you can access for free at:
<http://cebp.aacrjournals.org/content/11/12/1645.full#ref-list-1>

Citing articles This article has been cited by 5 HighWire-hosted articles. Access the articles at:
<http://cebp.aacrjournals.org/content/11/12/1645.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cebp.aacrjournals.org/content/11/12/1645>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.