

Inhibitory Effect of Glycolic Acid on Ultraviolet-Induced Skin Tumorigenesis in SKH-1 Hairless Mice and Its Mechanism of Action

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Glycolic acid, an alpha-hydroxy acid derived from fruit and milk sugars, has been used commonly as a cosmetic ingredient since it was discovered to have photoprotective and anti-inflammatory effects and antioxidant effects on ultraviolet (UV)B-irradiated skin. Little is known, however, about the functional role of glycolic acid on UV-induced skin tumorigenesis. In the present study, we examined the effect of glycolic acid on UV (UVA + UVB)-induced skin tumorigenesis and assessed several significant contributing factors in SKH-1 hairless mice. Inbred hairless female mice (15 animals/group) were irradiated for 5 d/wk at a total dose of 74.85 J/cm² UVA and 2.44 J/cm² UVB for 22 wk. Glycolic acid was applied topically twice a week at a dose of 8 mg/cm² immediately after UV irradiation. Glycolic acid reduced UV-induced skin tumor development. The protective effect of glycolic acid was a 20% reduction of skin tumor incidence, a 55% reduction of tumor multiplicity (average number of tumors/mouse), and a 47% decrease in the number of large tumors (larger than 2 mm). Glycolic acid also delayed the first appearance of tumor formation by about 3 wk. The inhibitory effect of glycolic acid on UV-induced tumor development was accompanied by decreased expression of the following UV-induced cell-cycle regulatory proteins: proliferating cell nuclear antigen (PCNA), cyclin D1, cyclin E, and the associated subunits cyclin-dependent kinase 2 (cdk2) and cdk4. In addition, the expression of p38 kinase, jun N-terminal kinase (JNK), and mitogen-activated protein kinase kinase (MEK) also was lower in UV + glycolic acid-treated skin compared with expression in UV-irradiated skin. Moreover, transcription factors activator protein 1 (AP-1) and nuclear factor κ B (NF- κ B) activation was significantly lower in UV + glycolic acid-treated skin compared with activation in UV-irradiated skin. These results show that glycolic acid reduced UV-induced skin tumor development. The decreased expression of the cell-cycle regulatory proteins PCNA, cyclin D1, cyclin E, cdk2, and cdk4 and the signal mediators JNK, p38 kinase, and MEK may play a significant role in the inhibitory effect of glycolic acid on UV-induced skin tumor development. In addition, the inhibition of activation of transcription factors AP-1 and NF- κ B could contribute significantly to the inhibitory effect of glycolic acid. © 2001 Wiley-Liss, Inc.

Key words: glycolic acid; ultraviolet; skin tumor; cyclins; cyclin-dependent kinase; mitogen-activated protein kinase family; transcription factors

INTRODUCTION

Ultraviolet (UV) light has been recognized as a complete carcinogen, responsible for both initiation and promotion of skin carcinogenesis [1]. Several studies have shown that UVB is the most effective wavelength in terms of causing skin tumor development. UVA is the most abundant wavelength in the solar spectrum, and it, too, has been found to be associated with skin tumor development [2–5]. In addition, several lines of evidence indicated that changes in the expression of cell-cycle regulatory proteins and the activation of transcription factors play important roles in UV-induced skin tumor development [6–9]. Furthermore, the mitogen-activated protein kinase (MAPK) family, conveying UV-induced signaling from the cell membrane to the nucleus, also is known to be a critical regulator

of the activation of various transcription factors as well as an important mediator of signals in skin tumor development [10–13].

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Abbreviations: UV, ultraviolet; SDS, sodium dodecyl sulfate; PCNA, proliferating cell nuclear antigen; cdk, cyclin-dependent kinase; MAPK, mitogen-activated protein kinase; JNK, jun N-terminal kinase; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; HRP, horseradish peroxidase; EMSA, electrophoretic mobility shift assay; AP-1, activator protein 1; NF- κ B, nuclear factor κ B.

Glycolic acid, an alpha-hydroxy acid derived from fruit and milk sugars, has been used commonly as a cosmetic ingredient [14,15]. Recently, alpha-hydroxy acid-containing cosmetics have gained public interest with their supposed ability to reduce wrinkles, roughness, age spots of the skin, and other forms of skin damage [16,17]. Photoprotective and anti-inflammatory effects, along with antioxidant effects, of topical glycolic acid treatment on UVB-irradiated skin also have been reported [18–20]. It is therefore possible that glycolic acid has a preventive effect on photocarcinogenesis. Indeed, anti-inflammatory and antioxidant effects have been implicated in preventive strategies against photocarcinogenesis and chemical carcinogenesis [1,3,5]. On the other hand, excessive and long-term use of these products can cause skin redness, swelling, and darkening.

Moreover, one study has shown that glycolic acid increases UV-induced sunburn cell formation and reduces the minimal erythema dose in normal human skin [21]. For this reason, speculation has arisen that these products can cause phototoxicity and carcinogenesis if used over a long period by people who are especially sensitive to sunlight [21]. To clarify these conflicting reports, we investigated the effect of glycolic acid on UV (UVA + UVB)-induced skin tumorigenesis. In addition, several possibly related mechanisms of action in photocarcinogenesis, that is, changes in the expression of cell-cycle regulatory proteins, proliferating cell nuclear antigen (PCNA), cyclins, cyclin-dependent kinases (cdks), and the MAPK family and changes in the activation of the transcription factors activator protein 1 (AP-1) and nuclear factor κ B (NF- κ B), also were examined in SKH-1 hairless mice.

MATERIALS AND METHODS

Experimental Animals

Pathogen-free hairless female SKH-1 mice (6 wk old) were obtained from the National Institute of Toxicological Research of the Korea Food and Drug Administration (Seoul, Korea). The animals were maintained in accordance with the guidelines of the National Institute of Toxicological Research of the Korea Food and Drug Administration for the care and use of laboratory animals. These animals were housed five per polycarbonate cage in a room that was temperature controlled at $22 \pm 2^\circ\text{C}$; they were given food and water ad libitum.

Animal Treatment and Tumorigenesis

A total of 45 mice were divided into three groups of 15 animals each. One experimental group received UV irradiation alone. The second group received UV irradiation plus glycolic acid applied topically. Control animals received glycolic acid

only. Thirty milligrams of glycolic acid (pH 3.0) in a cream base made with polyethylene glycol 400 and 8000 combination (1:2) was applied twice a week to the dorsal area ($1.5 \times 2.5 \text{ cm}^2$) of the skin. The amount of glycolic acid applied was 8 mg/cm^2 dorsal skin.

The mice were irradiated for 5 d/wk, beginning at a dose of 11.4 J/cm^2 UVA and 0.04 J/cm^2 UVB, for 22 wk, according to the methods of Pentland et al. [3]. The dose of light was then increased by 10% each week. The final cumulative doses were 74.85 J/cm^2 UVA and 2.44 J/cm^2 UVB. UV irradiation was carried out with a UV light (RMX 3 W; Dong Sung Laboratory Technology, Seoul, Korea) equipped with an F40T10 BLB 40W UVA lamp emitting maximally 365 nm and an F 40M UVB lamp emitting maximally 321 nm (Vilbert-Lourmat, Merne-La-Vallee, France). The radiation intensity was monitored with a VLX-3W radiometer (Vilbert-Lourmat) equipped with VLX-365 (UVA) and VLX-312 (UVB) sensors. The papillomas appearing on the skin were recorded every week during the experimental period. At 24 h after final treatment, animals were killed for histologic and biochemical analysis. The areas affected by skin tumors were fixed with buffered 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathologic evaluation.

Western Blotting

Skin tissue (0.3 g) was homogenized with lysis buffer (50 mM Tris at pH 8.0, 150 mM NaCl, 0.02% sodium azide, 1% sodium dodecyl sulfate (SDS), 100 $\mu\text{g/mL}$ phenylmethylsulfonyl fluoride (PMSF), 1 $\mu\text{L/mL}$ of aprotinin, 1% igapel 630 (Sigma Chemical Co., St. Louis, MO), and 0.5% deoxychoate) and centrifuged at $23\,000\times g$ for 1 h. Equal amounts of protein were separated on an SDS-12% polyacrylamide gel and transferred to nitrocellulose membranes (Hybond ECL; Amersham Pharmacia Biotech Inc., Piscataway, NJ). The blots were blocked for 2 h at room temperature with 5% (wt-vol) nonfat dry milk in Tris-buffered saline solution (10 mM Tris at pH 8.0 and 150 mM NaCl) containing 0.05% Tween-20. The membrane was incubated for 3 h at room temperature with specific antibodies.

Rabbit polyclonal antibodies against cyclin A, cyclin D1, cyclin E, cdk2, cdk4, cdk6, mitogen-activated protein kinase kinase (MEK), extracellular signal-regulated kinase (ERK), p38 kinase, jun N-terminal kinase (JNK), PCNA, and inhibitory κ B (I κ B) goat polyclonal antibody for c-fos; and mouse monoclonal antibody for c-jun (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were used in this study. The antibodies were used at dilutions specified by the manufacturer. The blots were incubated with the corresponding conjugated anti-rabbit, -goat (for c-fos), or -mouse (for c-jun) immunoglobulin G/horseradish peroxidase (HRP) (Santa Cruz

Biotechnology, Inc.). Detection of immunoreactive proteins was performed with the ECL Western blotting detection system. The relative densities of the protein bands were quantified by densitometry with the Electrophoresis Documentation and Analysis System 120 (Eastman Kodak, Rochester, NY).

Nuclear Extract and Electrophoretic Mobility Shift Assay

Preparation of nuclear extracts, nuclear extraction, and electrophoretic mobility shift assay (EMSA) were performed as described by Hong and Glauert [22], with minor modifications. Briefly, 0.4 g of skin was chopped into 1.5 mL of ice-cold phosphate-buffered saline (pH 7.6). The skin pieces were then homogenized and centrifuged at $12\,000\times g$ for 5 min at 4°C. The pellet was resuspended in 1000 μ L of cold buffer containing 10 mM HEPES (pH 7.9), 2 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol, and 0.1 mM EDTA and PMSF. After homogenization in a tight-fitting Dounce homogenizer, the homogenates were left on ice for 10 min and then centrifuged at $25\,000\times g$ for 10 min. The nuclear pellet was resuspended in 70 μ L of a second buffer containing 10 mM HEPES (pH 7.9), 300 mM NaCl, 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.1 mM PMSF, and 10% glycerol. The suspension was gently shaken for 20 min at 4°C.

After centrifugation at $25\,000\times g$ for 10 min, the nuclear extracts (supernatants) were collected and quickly frozen at $-80^\circ C$. EMSA was then performed. Briefly, 5 μ g of nuclear protein was incubated on ice for 15 min with reaction buffer containing 0.1 μ g/ μ L poly dI-dC, 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 μ g/ μ L bovine serum albumin, and 10% (vol-vol) glycerol. The ^{32}P -labeled double-stranded AP-1 and NF- κ B binding oligonucleotides (more than 10^5 cpm) were added to the reaction mixture for an additional 20 min on ice. For experiments involving supershift analysis, 2–4 μ g of polyclonal antibodies against different subunits of NF- κ B and AP-1 (Santa Cruz Biotechnology Inc.) were incubated with nuclear proteins for 30 min on ice before addition of ^{32}P -labeled probes. All the reaction mixtures were resolved on 6% polyacrylamide gels. The gels were vacuum-dried and exposed to Kodak MR films at $-80^\circ C$ overnight.

Statistics

The data were expressed as the means $\pm$ standard deviations. The statistical significance of the differences in the number of tumors per mouse and in the percentage of tumor-bearing mouse between UV-treated and UV + glycolic acid-treated groups was evaluated by the Student *t* test at 1-wk intervals. Other data (densitometric values) were analyzed with a one-way analysis of variance test, followed by Bonferroni adjustment as a post hoc test.

RESULTS

Effect of Glycolic Acid on UV-Induced Tumor Development

The body weight changes and food intake of the mice were observed for apparent toxic signs of UV and glycolic acid. Body weight gains in UV-treated and UV + glycolic acid-treated animals were similar to that of control animals for the first 9 wk. After 9 wk, the animals in the two treated groups showed a slightly reduced average body weight compared with control animals (less than 7% in average weight; data not shown). Food consumption was not different among the groups for the experimental period. These data indicated that the doses of each agent might not have been toxic to the animals.

Tumor incidence (percentage of mice bearing tumors), tumor multiplicity (average number of tumors/mouse), and tumor size were determined every week. Each tumor was confirmed by histopathologic examination. Tumors first appeared in the UV-treated animals after treatment for 9 wk and continued to appear throughout the experimental period. UV treatment caused 100% tumor (papilloma) incidence at 20 wk. Tumors first appeared in UV + glycolic acid-treated animals after 12 wk, and this group had a tumor incidence of 80% or less throughout the experimental period (Figure 1A). The average number of tumors at the termination of the study was 4.6 tumors per mouse in the UV-treated group and 2.6 tumors per mouse in the UV + glycolic acid-treated group (Figure 1B). Moreover, glycolic acid reduced the number of large tumors (larger than 2 mm) by about 47% (Table 1).

Effect of Glycolic Acid on UV-Induced Expression of Cell-Cycle Regulatory Proteins

Our preliminary data did not show that glycolic acid had any anti-inflammatory or antioxidant effects in mice and guinea pigs treated for 2 wk (unpublished data). For this reason, our present study focused on the expression of cell-cycle regulatory proteins, because such expression has been implicated in photocarcinogenesis. Expression of PCNA, cyclins, and cdks in UV-treated skin and UV + glycolic acid-treated skin was compared. The expression of cyclin D1 and E protein was elevated in UV-treated skin compared with expression in control skin. UV-induced elevation of the expression of cyclin E, but not D1, was inhibited in UV + glycolic acid-treated skin (Figure 2). In addition, expression of cdk2 and cdk4 was elevated in UV-treated skin, and the elevation of expression was inhibited by UV + glycolic acid treatment (Figure 2). Expression of the cell proliferation marker protein PCNA also was elevated in UV-treated skin compared with levels in control animals. Reduction of UV-induced elevation of PCNA expression

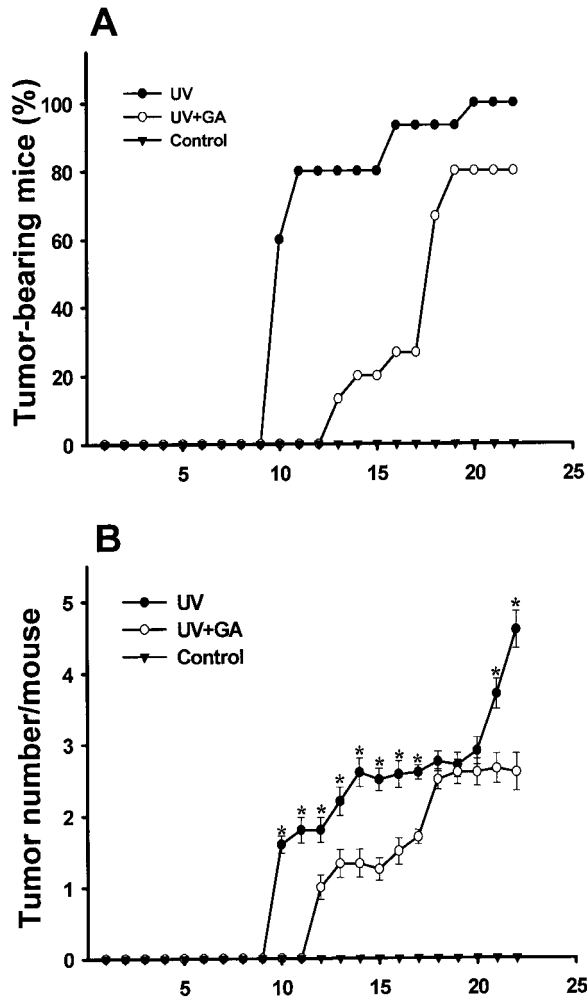


Figure 1. Tumor yield (A) and the number of tumors/mouse (B) in long-term UV-irradiated SKH-1 hairless mice. Fifteen mice in each group were irradiated with UV alone 5 d/wk for 22 wk (UV group) or treated with glycolic acid (8 mg/cm²) immediately after UV irradiation twice a week, as described in Materials and Methods. Asterisks indicate significant differences from the UV-irradiated group ($P < 0.05$). Control, glycolic acid-treated group; UV, ultraviolet-treated group; UV + GA, ultraviolet + glycolic acid-treated group.

Table 1. Skin Tumor Development by UV and UV + Glycolic Acid Treatment*

	UV	UV + glycolic
Number of tumors per group	69 ± 5	39 ± 3 [†]
Number of tumors < 2 mm in size	27 ± 2	20 ± 3
Number of tumors > 2 mm in size	42 ± 3	19 ± 1 [†]

*Data are expressed as the mean ± SD (n = 15 per group).

[†]Significantly different from UV-irradiated group ($P < 0.05$).

was observed in UV + glycolic acid-treated skin (Figure 2).

Effect of Glycolic Acid on UV-Induced Expression of MAPK Family and MEK

The MAPK family, including ERK, JNK, and p38 kinase, and upstream MEK expression have been associated with UV-induced signals that could consequently regulate the cell cycle and thereby influence skin tumor development [11,12]. For this reason, we investigated changes in the expression of the MAPK family (JNK, ERK, and p38 kinase) and upstream MEK. The expression of JNK, p38 kinase, and MEK was elevated in UV-treated skin compared with expression in untreated control skin. UV-induced elevation of expression of these proteins was inhibited in UV + glycolic acid-treated skin, but ERK expression was not different (Figure 3).

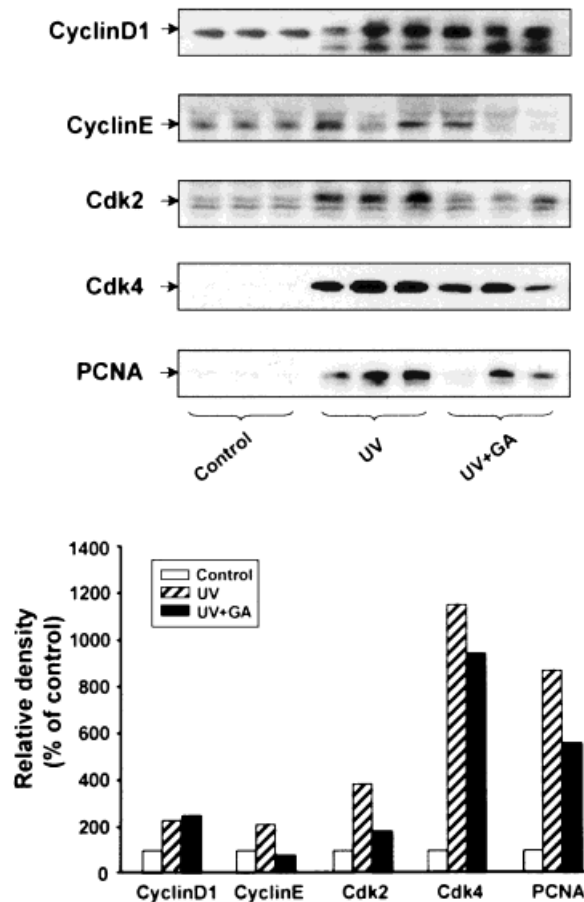


Figure 2. Expression of cyclins, cdks, and PCNA in UV-treated and UV + glycolic acid-treated skin. Three mice per each group were selected randomly and killed 24 h after final treatment. The epidermis protein was isolated and prepared for Western blotting analysis with appropriate primary antibodies and secondary HRP conjugates, as described in Materials and Methods. Densitometric quantification of protein content was performed with two different sets of Western blotting. Control, glycolic acid-treated group; UV, ultraviolet-treated group; UV+GA, ultraviolet+glycolic acid-treated group.

Effect of Glycolic Acid on UV-Induced Activation of Transcription Factors

The binding activities of transcription factors AP-1 and NF- κ B in UV-treated skin and UV + glycolic acid-treated skin were compared, to determine whether changes in the expression of MAPK and MEK could result in changes in the activation of transcription factors. The DNA-binding activity of AP-1 was elevated significantly in UV-treated skin compared with activity in control animals, but the binding activity was reduced in UV + glycolic acid-treated skin (Figure 4A). To confirm that the attenuated activity of AP-1 was due to the reduction of expression of c-jun or c-fos, the expression of these proteins was determined by Western blotting. c-fos expression was elevated in UV-treated skin, and elevation was inhibited in UV + glycolic acid-treated skin; however, c-jun expression was not changed (Figure 4B). As with AP-1 activation, an increase in NF- κ B activity was noted in UV-treated skin, but it was much higher. The increase in NF- κ B activation was inhibited by UV + glycolic acid treatment (Figure 5A). To determine the possible relevance of release of I κ B in the cytosol and

elevation of NF- κ B activity, the expression of I κ B protein in the cytosol was examined. Contrary to our expectations but consistent with the expression pattern of other proteins, I κ B expression was higher in UV-treated skin, and the elevation was reduced in UV + glycolic acid-treated skin (Figure 5B).

DISCUSSION

In this study, we found that glycolic acid had inhibitory effects against UV-induced skin tumor development in SKH-1 hairless mice. The observed inhibitory effects of glycolic acid were in accord with the decreased expression of PCNA, cyclins, cdk, the MAPK family members and JNK p38 kinase, and upstream kinase MEK. Moreover, glycolic acid attenuated the UV-induced activation of the transcription factors AP-1 and NF- κ B.

Glycolic acid is an alpha-hydroxy acid derived from fruit and milk sugars and has been used commonly as a cosmetic ingredient to reduce wrinkles, roughness, age spots, and other forms of skin damage [14–17]. It has been reported that glycolic acid has photoprotective, anti-inflammatory, and antioxidant effects on UVB-irradiated skin [18–20]. To our knowledge, however, this is the first report showing that glycolic acid has an inhibitory effect on UV-induced skin tumor development. Glycolic acid reduced tumor numbers per mouse and the percentage of tumor-bearing mice by the end of the experiment. Moreover, glycolic acid decreased the number of large tumors (larger than 2 mm). The mechanism of action by which glycolic acid inhibited UVB-induced tumorigenesis is not entirely understood. Anti-inflammatory and antioxidant effects of various agents generally have been recognized to be preventive mechanisms in photocarcinogenesis and chemical carcinogenesis.

Our preliminary data, however, did not show that treatment with varying doses of glycolic acid for 2 wk protects against a UV-induced increase of COX-2 expression, production of prostaglandin E₂, and lipid peroxidation in guinea pigs and mice (unpublished data). Therefore, our present investigation focused on determining the effect of glycolic acid on the expression of proteins related to the cell cycle and cell proliferation in the processes of UV-induced skin tumor development. It has been found that glycolic acid modifies epidermal cell proliferation *in vivo* and in cultured keratinocytes [23,24]. In addition, dysregulation of cell proliferation has become apparent in tumorigenesis. Cyclins, which are associated with the activation of cdk, control the progression of the cell cycle, which plays a central role in cell proliferation in several types of experimental animal and human tumors [6,7,25]. Moreover, up-regulation of the expression of cyclins and cdk proteins has been found in UV-induced papilloma and carcinoma [6,7]. Therefore, we first investigated whether an inhibitory effect of glycolic

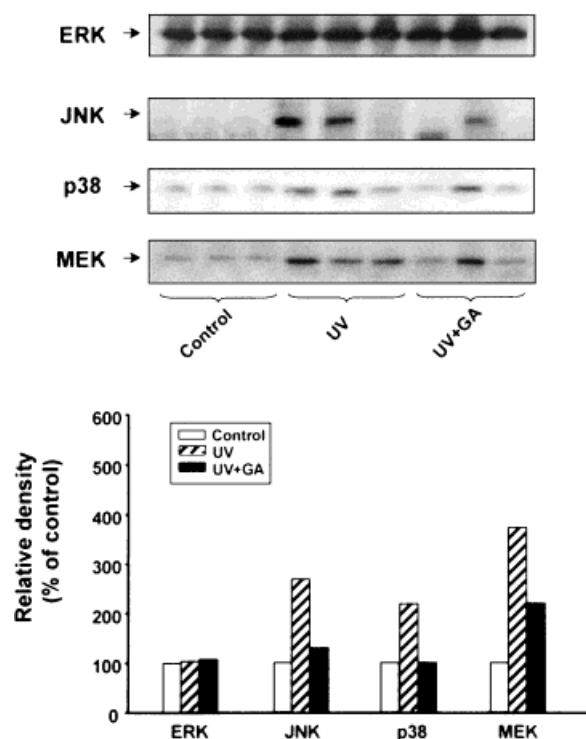


Figure 3. Expression of the MAPK family and MEK in UV-treated and UV + glycolic acid-treated skin. Three mice per each group were selected randomly and killed at 24 h after final treatment. The epidermis protein was isolated and prepared for Western blotting analysis with appropriate primary antibodies and secondary HRP conjugates, as described in Materials and Methods. Densitometric quantification of protein content was performed with two different sets of Western blotting. Control, glycolic acid-treated group; UV, ultraviolet-treated group; UV+GA, ultraviolet + glycolic acid-treated group.

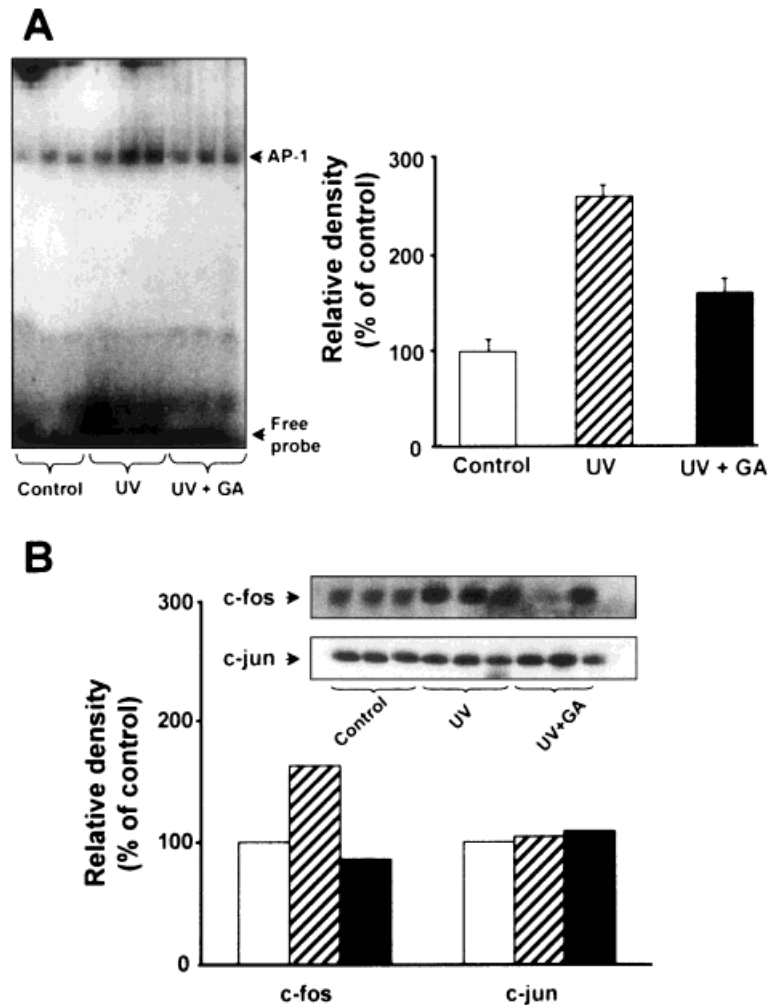


Figure 4. DNA-binding activity of transcription factor AP-1 (A) and expression of c-jun and c-fos (B). DNA-binding specificity was determined by incubation of 10 μ g of nuclear protein from the skin of three randomly selected mice 24 h after final treatment with 32 P-labeled AP-1 consensus double-stranded oligonucleotide probes, as described in Materials and Methods. Densitometric quantification

was performed on three assays. Expression of c-jun and c-fos proteins in UV-treated and UV + glycolic acid-treated skin was determined by Western blotting. Control, glycolic acid-treated group; UV, ultraviolet-treated group; UV+GA, ultraviolet + glycolic acid-treated group.

acid on UV-induced expression of cell-cycle regulatory proteins could result in reduction of tumor development.

In our study, expression of cyclin D1, cyclin E, cdk2, and cdk4 was elevated in UV-treated skin. On the other hand, elevation of cyclin E, cdk2, and cdk 4 expression was inhibited in UV + glycolic acid-treated skin. Expression of the proliferation marker protein PCNA was also elevated in UV-treated skin, and this elevation was inhibited by UV + glycolic acid treatment. The present data showed that the inhibitory effect of glycolic acid on the expression of the cell-cycle regulatory proteins cyclin D1, cyclin E, cdk2, and cdk4 could play an important role in the reduction of UV-induced skin tumor development. It has been reported that the well-known chemopreventive agents vitamin E and

green tea polyphenol fraction have inhibitory effects on UV-induced cell-cycle progression and thereby inhibit tumor formation [26,27].

The next stage of our study focused on determining whether the inhibitory effect of glycolic acid could be related to down-regulation of the signal-mediator MAPK family proteins and upstream MEK, which have been shown to mediate UV-induced signals in the processes of tumor development. The expression of JNK, p38 kinase, and upstream MEK in UV-treated skin was elevated, whereas elevation was inhibited in the UV + glycolic acid-treated skin. There are three subtypes of MAPKs: ERK, p38 kinase, and JNK. MAPKs are known to play a significant role in the regulation of cell proliferation in skin, because they convey UV-induced signals from the cell membrane to nucleus, where many transcrip-

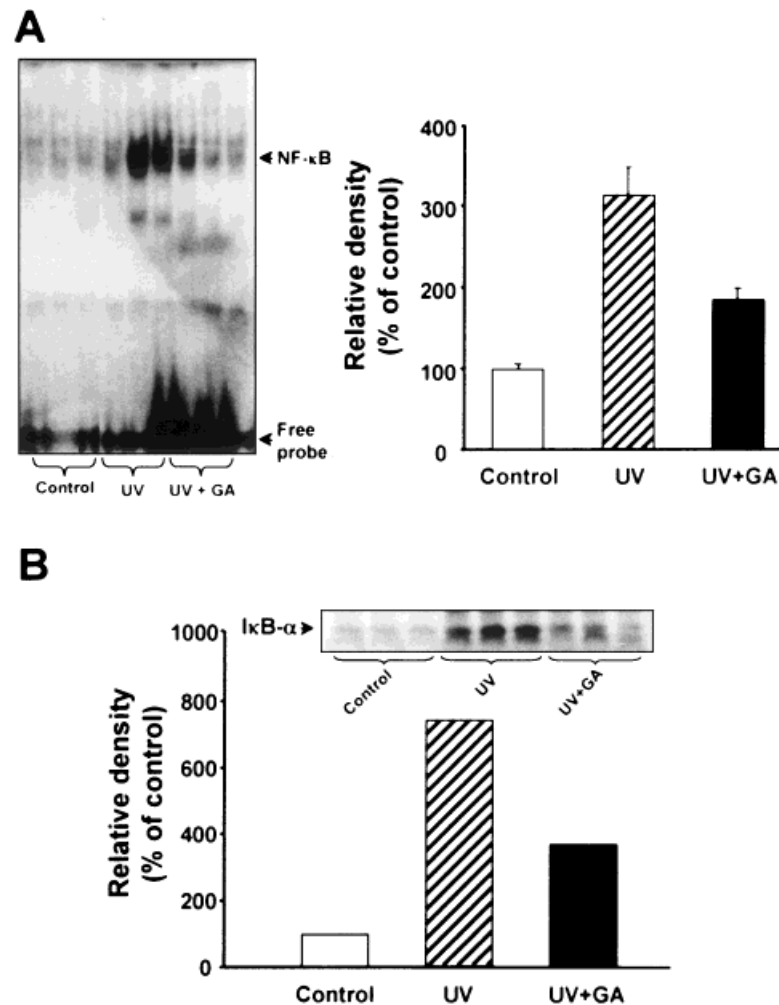


Figure 5. DNA-binding activity of transcription factors NF-κB (A) and expression of IκB in the cytosol (B). DNA-binding specificity was determined by incubation of 10 μg of nuclear protein from the skin of three randomly selected mice at 24 h after final treatment with ³²P-labeled NF-κB consensus double-strand oligonucleotide probes,

as described in Materials and Methods. Densitometric quantification was performed with three assays. Expression of IκB in the UV-treated and UV+glycolic acid-treated skin was determined by Western blotting. Control, glycolic acid-treated group; UV, ultraviolet-treated group; UV+GA, ultraviolet with glycolic acid-treated group.

tion factors are located in [28]. Moreover, MAPKs, as well as MEK, are known to modify the activation of the cell proliferation regulatory transcription factors NF-κB and AP-1 [29,30].

In the present study, the expression of JNK, p38 kinase, and MEK, but not JNK, was increased in UV-treated skin, and glycolic acid attenuated these elevations in expression. It is not clear which signal pathways are most involved in the processes of UV-induced skin tumor development and could be blocked by glycolic acid *in vivo*. It is worth noting, however, that depending on the stimulators used, different solar UV lights, and skin cell types, differential activation of these kinases has been observed [31–33]. The tumor-preventive agent silymarin has been found to modulate JNK and ERK, but not p38 kinase, expression in carcinoma

cells, whereas 5-aminolevulinate-photodynamic activates JNK and p38 kinase, but not ERK, in cultured HaCaT cells [11,33]. The present data also showed that inhibition of the MEK/JNK and/or p38 kinase-mediated pathways may play a role in the inhibition of expression of cell-cycle regulatory proteins, which may lead to inhibition of UV-induced tumor development. Further study is needed to establish which signal pathways are most involved in the inhibitory effect of glycolic acid on UV-induced tumor development.

To investigate the possible mechanism of the inhibitory effect of glycolic acid further, we next examined the change in activation of the transcription factors AP-1 and NF-κB. AP-1 and NF-κB have been considerably implicated in the control of cell growth and oncogenesis. It has been found that

activation of NF- κ B and AP-1 promotes proliferation and cellular transformation [34,35]. Moreover, recent evidence also has shown that AP-1 and NF- κ B are activated in the process of skin tumor development, and several antitumor agents have been found to inhibit UV-induced activation of AP-1 and NF- κ B in vivo and in vitro [36–39]. Furthermore, it has been reported that NF- κ B has a role in epithelial cell growth arrest [40]. In the present investigation, activation of AP-1 and NF- κ B also was increased significantly in UV-treated skin compared with control skin. The increase in AP-1 and NF- κ B activation was attenuated in UV + glycolic acid-treated skin. In conjunction with the activation of AP-1, the expression of c-fos, a subunit of AP-1, but not c-jun, was increased significantly in UV-treated skin, and glycolic acid inhibited its expression. These data suggest that a change in c-fos protein, rather than c-jun expression or other mechanisms of action, may play the largest role in the activation of AP-1.

Consistent with the changes in the activation of NF- κ B, the expression of I κ B in the cytosol was increased in UV-treated skin and was reduced with UV + glycolic acid treatment. This finding suggested that the release of I κ B was elevated and thus led to an increase in the translocation of NF- κ B to the nucleus, where it could be activated. It is not clear why I κ B expression was still high in the cytosol without degradation. It is generally recognized that it should be degraded soon after release from NF- κ B via phosphorylation of I κ B at the N-terminal serine residue. It is worth noting that phosphorylation of I κ B at the tyrosine residue could cause dissociation of I κ B from NF- κ B without degradation [41,42]. Further study must be undertaken to clarify this issue. These data show that inhibition of the activation of transcription factors AP-1 and NF- κ B also may be involved in the reduction of UV-induced tumor development by glycolic acid.

Collectively, the inhibitory effects of glycolic acid on the expression of cell-cycle regulatory proteins and on the activation of mitogenic signal molecules could be involved in the prevention of UV-induced carcinogenesis. The experimental dose in our study was about 10 times higher than the daily amount used typically by the public (1 mg/cm² glycolic acid applied twice a day). Glycolic acid was applied twice a week to mice, in contrast to twice-a-day use by the public. Therefore, the experimental dose of glycolic acid (8 mg/cm²) used in our study was approximately similar to the dose used by the public.

The present study thus showed that clinical doses of glycolic acid may be beneficial in terms of photocarcinogenesis. We are presently investigating the anti-inflammatory and antioxidant effects of glycolic acid in photocarcinogenesis. The dose-related effects of glycolic acid on the processes of UV-induced skin tumor development are also under

investigation to determine whether glycolic acid has an enhancing effect on photocarcinogenesis at high doses. In summary, this study showed that glycolic acid reduces UV-induced skin tumor development. In addition, these results show that glycolic acid inhibits UV-induced expression of cell cycle regulatory proteins and signal mediator proteins as well as activation of transcription factors, which could contribute significantly to the inhibitory effect of glycolic acid in the photocarcinogenesis.

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