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甘草酸與其衍生物對保護紫外線照射引起的皮膚老化與皮膚癌的分子機制(第 3 年)

研究成果報告(完整版)

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Molecular Mechanisms Underlying Chemopreventive Activities of Glycyrrhizic Acid against UVB-Radiation-Induced Carcinogenesis in SKH-1 Hairless Mouse Epidermis

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Molecular Mechanisms Underlying Chemopreventive Activities of Glycyrrhizic Acid against UVB-Radiation-Induced Carcinogenesis in SKH-1 Hairless Mouse Epidermis

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Glycyrrhizic acid has been shown to possess anti-inflammation, antiviral and chemoprotective activity against tumors. We evaluated the protective effects of glycyrrhizic acid in UVB-radiation-induced skin tumor formation in SKH-1 hairless mice and the early molecular biomarkers of these effects. Mice irradiated at 180 mJ/cm² twice per week showed 100% tumor incidence in 20 weeks. Feeding with glycyrrhizic acid prior to UVB irradiation caused delays in tumor appearance, multiplicity and size. Feeding with glycyrrhizic acid for 2 weeks before a single UVB irradiation (180 mJ/cm²) resulted in significant decrease in UVB-radiation-induced thymine dimer-positive cells, expression of proliferative cell nuclear antigen (PCNA), terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive cells, and apoptotic sunburn cells together with an increase in p53- and p21/Cip1-positive cell populations in epidermis. Simultaneously, glycyrrhizic acid also significantly inhibited NF-κB, cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), and nitric oxide (NO) levels. Thus glycyrrhizic acid ameliorates UVB-radiation-induced tumorigenesis via downregulation of cell proliferation controls involving thymine dimer, PCNA, apoptosis and transcription factor NF-κB and of inflammatory responses involving COX-2, PGE2 and NO while upregulating of p53 and p21/Cip1 to prevent DNA damage and facilitate DNA repair.

INTRODUCTION

Ultraviolet (UV) radiation present in sunlight is responsible for the majority of the damage to the skin. Exposure to UVB radiation and to a much lesser extent to UVA radiation is the major etiological factor leading to the development of cutaneous squamous and basal cell carcinoma (*1, 2*). Each year, approximately 1 million new cases of skin cancer are attributable to exposure to UV radiation (*3*). An aging population, increased recreational exposure to sunlight, and the depletion of the ozone layer are believed to be major factors contributing to the globally increasing incidence of skin cancer (*2*).

Experiments with a hairless mouse model of skin carcinogenesis showed the dependence of skin tumor development on the dose of and duration of exposure to UV radiation (*4*) and identified UVB radiation as the most mutagenic and carcinogenic region of the solar spectrum (*5*). Tumor development involves an imbalance of the endogenous antioxidant system, leading to an increase in free radical levels and inflammation. UVB radiation also causes direct DNA damage to exert its detrimental effect (*6, 7*). Thus UVB radiation is considered to be a complete carcinogen because it can initiate and induce cancer growth in the absence of any other carcinogen (*1*).

Glycyrrhizic acid has been shown to possess several beneficial biological activities, including inhibition of the mouse skin tumor-initiating activity of DMBA, anti-ulcerative effects, anti-inflammation, interferon induction and anti-hepatotoxic effects (*8–19*), and it is active against a range of viruses (*20–27*). Clinically, glycyrrhizic acid has been used to treat patients with chronic active hepatitis (*28–30*). In addition to having anti-inflammatory actions, glycyrrhizic acid also acts as a chemoprotective agent against tumors (*31*).

Our previous studies indicated that glycyrrhizic acid not only possesses antiviral activities (*21–22, 26*) but also protects against neuronal cell death resulting from glutamate excitotoxicity (*11*). We also presented evidence that the inhibitory effect of glycyrrhizic acid was

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TABLE 1
Experimental Design Depicting Variables and Treatment Groups in both Long-Term and Short-Term Study

Exposure	No glycyrrhizic acid	Glycyrrhizic acid feeding[a]
No UVB radiation	(I) (Control)	(II) (Glycyrrhizic acid)
UVB radiation[b]	(III) (UVB radiation)	(IV) (Glycyrrhizic acid + UVB radiation)

Notes. Mice were divided into four groups, (I), (II), (III) and (IV). Twenty mice were in each group in the long-term and five mice in each group for short-term study.
[a] Glycyrrhizic acid in drinking water.
[b] UVB irradiation (180 mJ/cm^2), twice/week for long-term and single exposure for short-term study.

mediated via blockade of NF-κB activation by interfering with a pathway that leads to the phosphorylation or degradation of IκB (*11*). In the present study, we extended these findings to examine the efficacy of glycyrrhizic acid in the reduction of UVB-radiation-induced skin tumor formation in hairless mice and the underlying molecular events of this efficacy.

MATERIALS AND METHODS

Animals, Chemical and UVB Light Source

Inbred female SKH-1 hairless mice (5 weeks old) were purchased from Charles River Laboratories (Wilmington, MA) and maintained in accordance with the relevant guidelines and regulations for the care and use of laboratory animals of China Medical University.

The UVB light source consisted of four FS-40-T-12-UVB sunlamps (Philips, Amsterdam, The Netherlands) that emitted ~80% radiation in the range of 280 to 340 nm with a peak emission at 314 nm as monitored with an SED 240 photodetector with SPS300 filter and a T input optic connected to an ILT1700 Research Radiometer (International Light Technologies, Newburyport, MA). The SPS300 filter removes wavelengths shorter than 280 nm, and the predominant emitting peak is at 280–315 nm. The radiometer is calibrated on a regular basis against both a traceable standard lamp and the laboratory radiation source.

Mice were exposed to UV radiation for 2 min and 40 s with a distance of 23 cm between the light source and the target skin.

A stock solution of glycyrrhizic acid (Acros Organics, Morris Plains, NJ) was made in phosphate-buffered saline, pH 7.4. For the feeding study, drinking water containing 0.02% glycyrrhizic acid was prepared.

Experimental Designs

Both long-term and short-term studies were conducted to assess the effect of glycyrrhizic acid on UVB-radiation-induced skin carcinogenesis in female SKH-1 hairless mice. The experimental designs, variables and treatment groups are shown in Table 1. The long-term regimen was designed to assess the effect of glycyrrhizic acid on UVB-radiation-induced skin tumor incidence, whereas the short-term study was for assessing the early molecular biomarkers.

Dorsal skin specimens were taken for immunohistochemical analyses at different times. Samples taken 1 h after UVB irradiation were used for the analysis of thymine dimer formation, the 8-h samples for p53, p21/Cip1 and proliferating cell nuclear antigen (PCNA) and the 12-h samples for deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and apoptotic sunburn cells. In addition, the cellular and nuclear proteins prepared from 8-h samples were used for NO, COX-2, PGE2 and NF-κB assays by ELISA.

Immunohistochemical Analysis of Biomarkers

Skin tissue sections from the mice in the short-term study were processed for immunohistochemical staining and biochemical analyses. To detect thymine dimer-positive cells, anti-thymine dimer antibody (Kamiya Biomedical Company, Seattle, WA) was used according to the manufacturer's protocol. Activities were then detected using a NovoLink Polymer Detection System (Novocastra Laboratories, Newcastle Upon Tyne, UK). For detection of p53, p21/Cip1 and PCNA, mouse monoclonal anti-p53 (LifeSpan BioSciences, Seattle, WA), anti-p21/Cip1 (Acris Antibodies GmbH, Herford, Germany), and anti-PCNA (Biocare Medical, Concord, CA) antibodies were used. Activities were detected using NovoLink Polymer Detection System (Novocastra Laboratories). The apoptotic cells were detected by using the DeadEnd Colorimetric TUNEL system (Promega Corporation, Madison, WI) and the apoptotic sunburn cells were stained conventionally with H&E and examined by light microscopy.

The microscopic examinations were performed by two investigators in a blind fashion. For every specimen, five to ten randomly selected fields were examined and counted at 400× magnification. Data were calculated as means ± SE of 25 fields/5 mice/group.

Biochemical Analysis of NO, COX-2 and PGE2 by ELISA

The frozen skin samples were pulverized in liquid nitrogen. The powder was suspended in cell lysis buffer (*11*) and sonicated before centrifugation at 12,500*g* for 20 min. The supernatants were used for quantification of NO (BioVision, Mountain View, CA), COX-2 (USCN LIFE, Wuhan, China), and PGE2 (R&D Systems, Minneapolis, MN), using ELISA kits following the manufacturer's protocols.

Analysis of NF-κB DNA-Binding Activity

For analyzing transcription factor NF-κB binding to DNA, nuclear proteins were prepared as described previously (*11*) and the binding activity was quantified using a TF ELISA kit (Panomics, Fremont, CA). This method is faster, easier and more sensitive than electrophoretic mobility shift assays and does not require the use of radioactivity. Briefly, the activated NF-κB p50 molecules from nuclear extracts bind to an NF-κB consensus binding site (NF-κB probe) on biotinylated oligonucleotides that were immobilized on a streptavidin-coated 96-well plate. The NF-κB p50 bound to the oligonucleotide is detected by an antibody directed against NF-κB p50. An additional HRP-conjugated secondary antibody provides a sensitive colorimetric readout quantified by spectrophotometry.

Statistical Analysis

Data are presented as means ± SE. The evaluation of statistical significance was determined by one-way analysis of variance (ANOVA) followed by Bonferroni *t* test for multiple comparisons. A *P* value less than 0.05 was considered statistically significant.

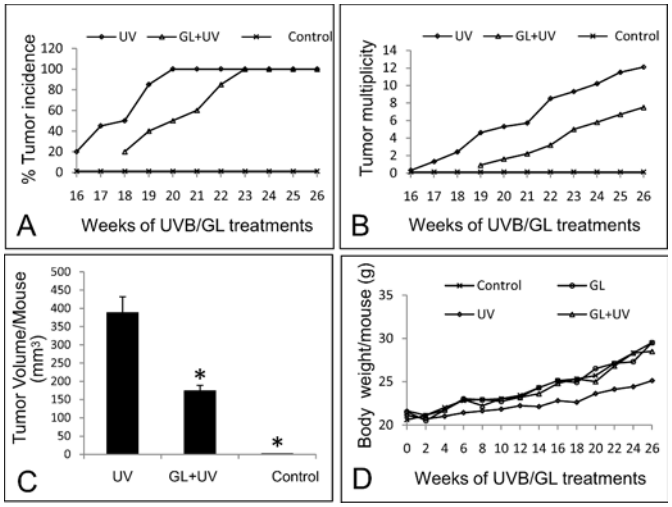

FIG. 1. Effect of glycyrrhizic acid (GL) on UVB-radiation-induced skin photocarcinogenesis in SKH-1 hairless mice. A long-term regimen was used for this study. Percentage of tumor incidence (panel A), tumor multiplicity per mouse (panel B), tumor volume per tumor per mouse (panel C), and body weight per mouse (panel D) were recorded and analyzed. The data shown in panel C are means ± SE. All data are from 20 mice in each group. No tumors were observed in the glycyrrhizic acid alone group.

RESULTS

Protective Effects of Glycyrrhizic Acid on UVB-Radiation-Induced Tumorigenesis in SKH-1 Hairless Mice

Exposure of mice to 180 mJ/cm^2 UVB radiation twice per week resulted in the development of skin tumors in all mice by 20 weeks; however, it took 23 weeks for the development of tumors in all mice in the oral glycyrrhizic acid treatment plus UVB radiation groups (Fig. 1A). The first tumor appearance in the UVB radiation alone animals occurred at the 16th week; this was delayed by 2 weeks in the glycyrrhizic acid-treated groups. Compared with the UVB radiation alone group (Fig. 1B), oral glycyrrhizic acid administration reduced the number of tumors per mouse throughout the experiment ($P < 0.001$). Tumor volume per tumor per mouse was also decreased from 388 ± 44 mm^3 in the UVB radiation alone group to 174 ± 15 mm^3 in the glycyrrhizic acid feeding group, a 55% decrease ($P < 0.005$) (Fig. 1C). No tumors were observed in the control and glycyrrhizic acid feeding groups (data not shown). None of the glycyrrhizic acid treatments caused any significant decrease in diet consumption (data not shown) or any body weight changes (Fig. 1D), indicating no observable toxicity in SKH1 mice.

Glycyrrhizic Acid Inhibits UVB-Radiation-Induced Apoptosis and Apoptotic Sunburn Cell

Compared with unexposed control mice (Fig. 2A), UVB-radiation exposure significantly increased TUNEL-positive apoptotic cells (Fig. 2B). Oral glycyrrhizic acid treatment without UVB irradiation (Fig. 2C) did not significantly induce apoptotic cells. However, oral administration of glycyrrhizic acid prior to UVB irradiation (Fig. 2D) significantly decreased the numbers of UVB-radiation-induced apoptotic cells. Quantitative data revealed that UVB irradiation resulted in 28.85 ± 2.62% of TUNEL-positive apoptotic cells, while low levels of such cells were observed in the unirradiated controls (2.75 ± 0.49) and the oral glycyrrhizic acid group (2.62 ± 0.25). In contrast, oral application of glycyrrhizic acid prior to UVB irradiation resulted in a significant reduction of TUNEL-positive apoptotic cells to 16.1 ± 2.3 ($P < 0.01$).

H&E staining (Fig. 2F) of apoptotic sunburn cells was markedly increased from 0.94 ± 0.16% in unexposed controls to 11.55 ± 1.63% in UVB-irradiated mice. Oral administration of glycyrrhizic acid prior to UVB irradiation resulted in a significant reduction of sunburn cells to 5.45 ± 0.92 ($P < 0.01$). Similar to the unexposed controls, low levels of sunburn cells were observed after oral glycyrrhizic acid treatment alone (1.03 ± 0.24).

Glycyrrhizic Acid Protects UVB-Radiation-Induced DNA Damage in SKH-1 Hairless Mouse Epidermis

Thymine dimers are considered as an early and important biomarker for UVB-radiation-induced DNA damage. A previous study showed that UVB-radiation-induced thymine dimer formation in the epidermis peaks at 1 h after irradiation (*32*). Compared with sham-irradiated controls (Fig. 3A), a single exposure of mice to UVB radiation strongly induced the formation of thymine dimer-positive cells (Fig. 3B). Glycyrrhizic acid feeding (Fig. 3C) resulted in a remarkably reduced thymine dimer-positive population. More intense staining for thymine dimers was observed in the suprabasal layer than in the basal layer. Fewer stained cells and less intense staining for thymine dimers were observed in the dermis than in the epidermis. Glycyrrhizic acid by itself in drinking water had no effect on biomarkers; therefore, the immunohistochemical staining pictures were not shown in this and other figures. Quantitative analysis (Fig. 3D) showed that exposure to UVB radiation resulted in 91 ± 9.9% thymine dimer-positive cells in epidermis, while negligible levels of these cells were observed in unirradiated controls or in mice treated with oral glycyrrhizic acid alone. In contrast, oral glycyrrhizic acid prior to UVB irradiation resulted in a significant reduction (35.8 ± 3.9%) in thymine dimer-positive cells ($P < 0.004$). These results suggest that glycyrrhizic acid could protect the epidermis against UVB-radiation-induced damage at least in part through suppression of thymine dimer formation.

Glycyrrhizic Acid Upregulates UVB-Radiation-Induced Activation of p53-p21/Cip1 Cascade

The unexposed and untreated control mice showed very low levels of p53-positive cells (Fig. 4A). Upon

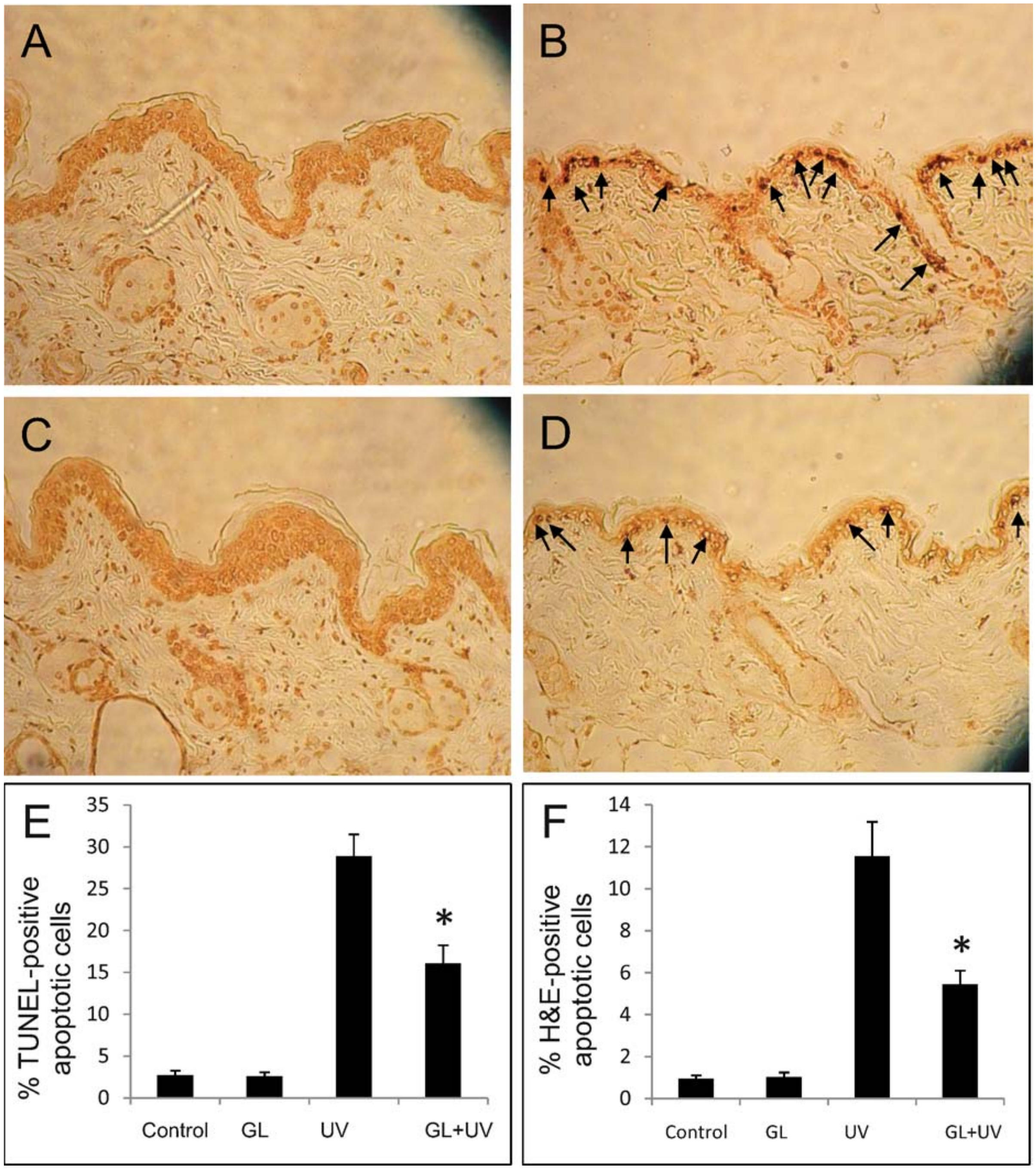

FIG. 2. Glycyrrhizic acid (GL) inhibits UVB-radiation-induced apoptosis and apoptotic sunburn cell formation. The apoptotic sunburn cells were detected by TUNEL assay and H&E staining. The results of TUNEL assay are shown as follows: panel A, control; panel B, UV radiation; panel C, glycyrrhizic acid; panel D, glycyrrhizic acid + UV radiation. Quantitative results of TUNEL-positive cells (panel E) and H&E-positive cells (panel F) were analyzed statistically. Data are shown as means ± SE of 25 fields/5 mice/group. *$P < 0.01$ for both TUNEL assay and sunburn cells.

UVB irradiation p53-positive cells were remarkably increased (Fig. 4B) and were seen primarily in the basal layer, but some were also observed in the suprabasal layer of the epidermis near the basal layer. Oral application of glycyrrhizic acid prior to UVB irradiation further enhanced the numbers of p53-positive cells (Fig. 4C). Consistent with these results, quantitative analyses revealed that exposure to UVB radiation resulted in 29 ± 3% p53-positive cells (Fig. 4D), which were further increased to 52 ± 5% in the glycyrrhizic acid plus UVB radiation group ($P < 0.02$), while low levels of such cells were observed in unirradiated controls (1.85 ± 0.21%) and mice given oral glycyrrhizic acid (1.9 ± 0.3%).

The same samples used for p53 detection were also analyzed for p21/Cip1-positive cells. Compared with

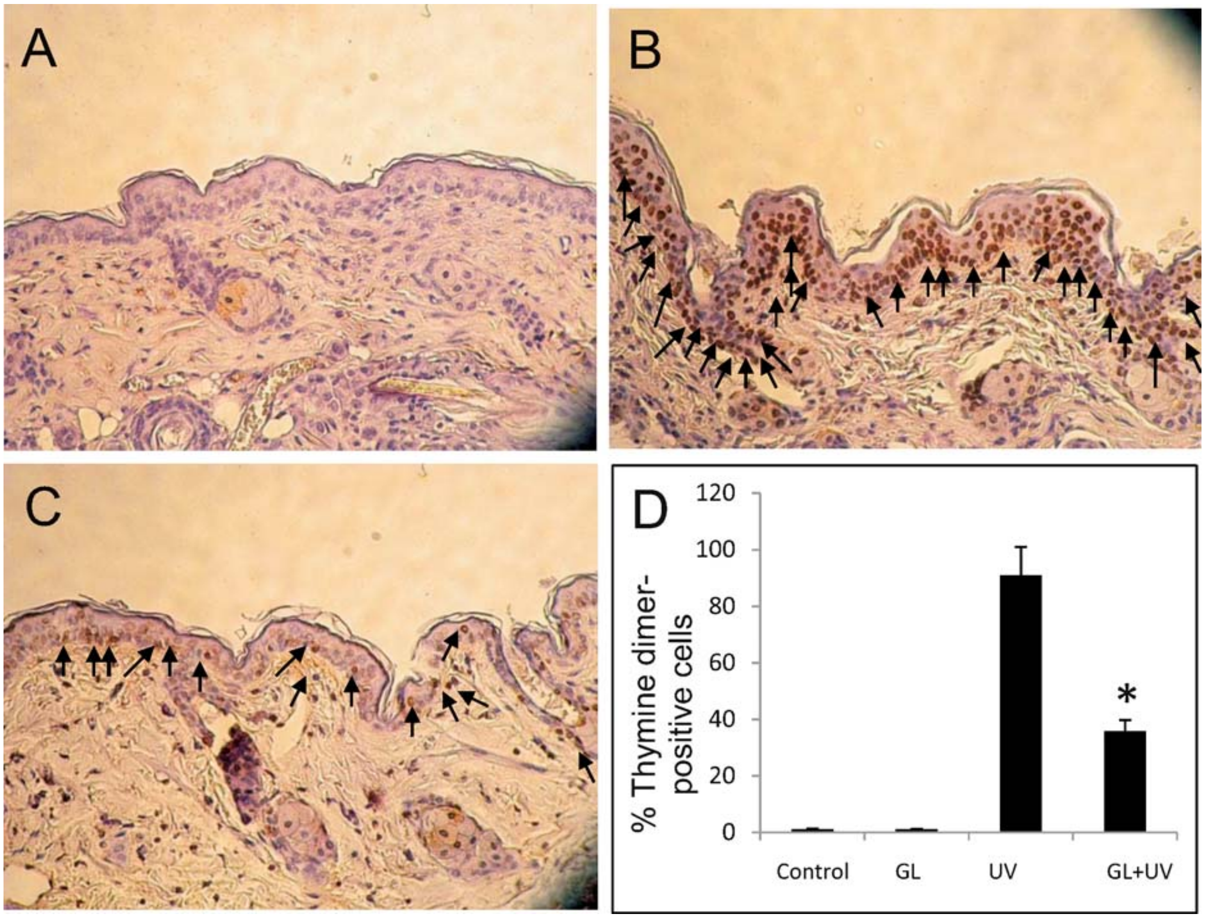

FIG. 3. Inhibition of UVB-radiation-induced thymine dimer-positive cells by glycyrrhizic acid (GL). Panel A, control; panel B, UV radiation; panel C, glycyrrhizic acid + UV radiation. Similar undetectable levels of thymine dimer-positive cells were observed in the glycyrrhizic acid alone group and unexposed controls (data not shown). Panel D shows the quantitative results for four different experimental conditions. Data are shown as means ± SE of 25 fields/5 mice/group. *$P < 0.004$.

unexposed control mice (Fig. 5A), UVB radiation alone significantly increased the p21/Cip1-positive cells (Fig. 5B). Oral administration of glycyrrhizic acid prior to irradiation significantly enhanced the numbers of p21/Cip1-positive cells (Fig. 5C). Exposure to UVB radiation resulted in 13.0 ± 0.7% p21/Cip1-positive cells in the epidermis, while low levels of such cells were observed in unirradiated controls (1.25 ± 0.07%) and after oral glycyrrhizic acid treatment (1.47 ± 0.09%) (Fig. 5D). In contrast, oral glycyrrhizic acid plus UVB radiation significantly increased p21/Cip1-positive cells to 28.2 ± 1.6% ($P < 0.003$).

Glycyrrhizic Acid Suppresses UVB-Radiation-Induced Cell Proliferation

We next examined the effect of UVB radiation without or with glycyrrhizic acid treatment on proliferation status of epidermis by measuring PCNA levels. Compared with unexposed control mice (Fig. 6A), UVB radiation alone remarkably increased the numbers of PCNA-positive cells (Fig. 6B). However, oral application of glycyrrhizic acid prior to UVB irradiation significantly decreased the numbers of PCNA-positive cells (Fig. 6C). Quantitative data revealed low levels of PCNA-positive cells in unirradiated controls (1.9 ± 0.1%) and after oral glycyrrhizic acid treatment (2.2 ± 0.1%). In contrast, UVB irradiation resulted in 28.1 ± 1.6% PCNA-positive cells in epidermis, which decreased to 19.8 ± 1.1% ($P < 0.009$) after glycyrrhizic acid plus UVB irradiation.

Glycyrrhizic Acid Inhibits UVB-Radiation-Induced Activation of NF-κB, COX-2, NO and PEG2 Levels

We next examined effects of UVB radiation without or with glycyrrhizic acid treatment on these cellular factors associated with skin tumorigenesis. Figure 7A shows that UVB radiation significantly increased the activity of NF-κB from 0.15 ± 0.01 in control mice and 0.16 ± 0.02 after oral glycyrrhizic acid alone to 0.58 ± 0.04. However, oral glycyrrhizic acid prior to irradiation significantly decreased the activity of NF-κB down to 0.28 ± 0.02 ($P < 0.003$).

Increased COX-2 activity (Fig. 7B) was observed in response to UVB irradiation (0.93 ± 0.06), but activity

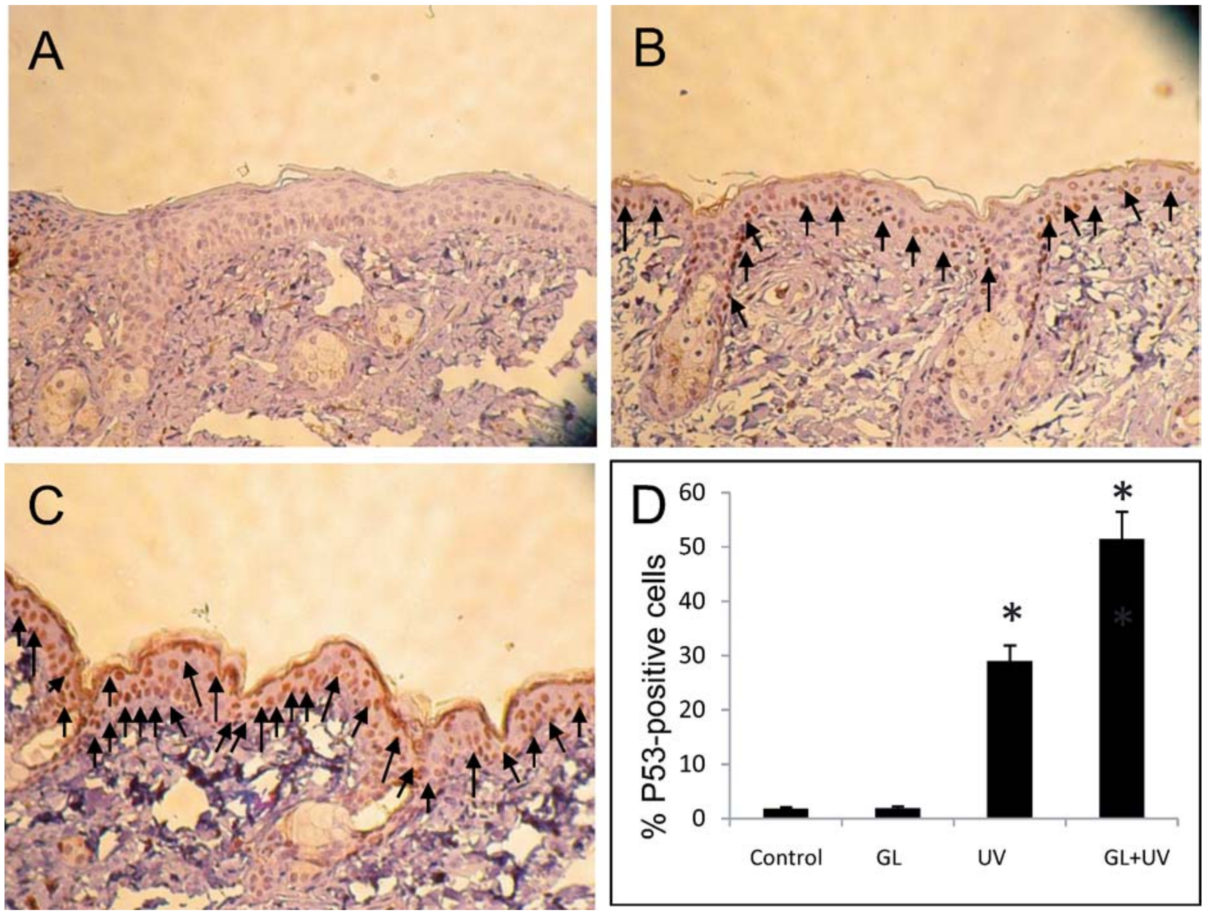

FIG. 4. Glycyrrhizic acid (GL) enhances UVB-radiation-induced p53 expression. Panel A, control; panel B, UV radiation; panel C, glycyrrhizic acid + UV radiation. Panel D shows the quantitative results for p53-positive cells for four different experimental conditions. $*P < 0.02$.

was significantly decreased by oral glycyrrhizic acid plus radiation (0.49 ± 0.04) ($P < 0.004$). Similar background levels of COX-2 activity were observed in unirradiated controls (0.35 ± 0.02) and mice receiving oral glycyrrhizic acid (0.32 ± 0.03).

Similarly, UVB-radiation exposure alone significantly increased the activity of NO to approximately 4.7-fold higher than that in unexposed controls (Fig. 7C). However, oral glycyrrhizic acid prior to irradiation significantly decreased the activity of NO ($P < 0.003$). PGE2 activity was markedly increased in response to UVB irradiation (Fig. 7D) but was significantly decreased by oral glycyrrhizic acid treatment ($P < 0.01$). Glycyrrhizic acid by itself did not affect the activity of NO and PGE2.

DISCUSSION

The major findings in the present study are that glycyrrhizic acid feeding caused a delay and reduction in UVB-radiation-induced tumor appearance, multiplicity and size in hairless mice without any toxicity. The photoprotective effect of glycyrrhizic acid could occur at several mechanistically different levels. Our results further showed that glycyrrhizic acid protects SKH-1 hairless mouse skin from UVB-radiation-induced DNA damage and that radiation-induced cell proliferation and apoptotic sunburn cell formation were prevented by glycyrrhizic acid possibly by further induction of the p53-p21/Cip1 cascade.

Sunburn cells are formed in the mammalian epidermis after exposure to UV radiation. These cells have a distinct morphology, with a shrunken, homogenized, densely staining cytoplasm and a hyperchromatic condensed pyknotic nucleus. In this study, these features were readily seen with routine H&E staining using light microscopy. It has been demonstrated that sunburn cells are apoptotic cells and contain a hallmark of apoptosis, namely DNA strand breaks observed by end labeling.

One of the most important characteristics of UV-radiation-induced carcinogenesis is DNA damage and mutagenesis, and thymine dimers are known as ‘‘hot spots’’ of UV-radiation mutagenesis (*32*). Our study showed that glycyrrhizic acid treatment markedly decreased in UV-radiation-induced thymine dimer-positive cells. Glycyrrhizic acid treatment may result in

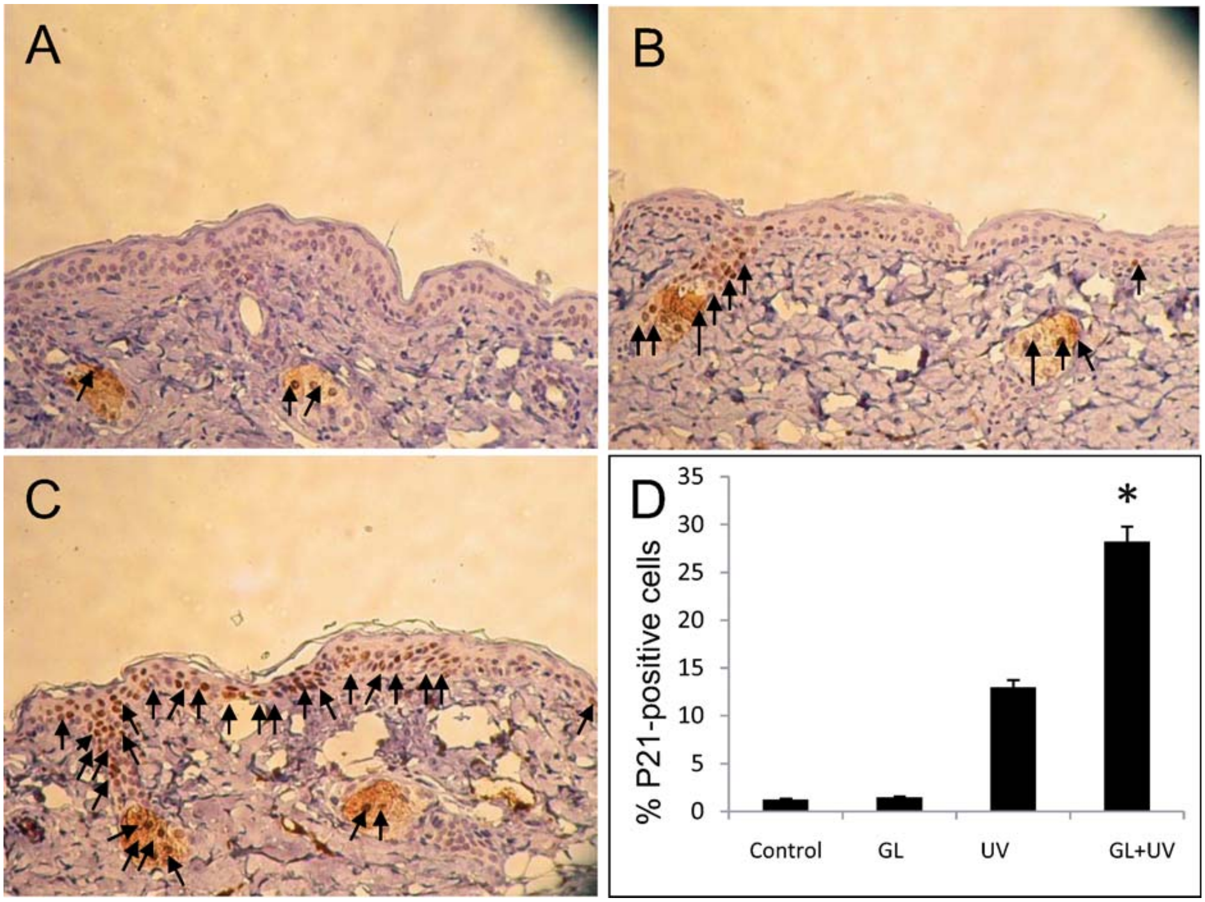

FIG. 5. Glycyrrhizic acid (GL) enhances UVB-radiation-induced p21/Cip1 expression. Panel A, control; panel B, UV radiation; panel C, glycyrrhizic acid + UV radiation. The quantitative results for p21 are shown in panel D. *$P < 0.003$.

an increase in the mismatch repair enzyme MSH2. A previous study showed that the DNA mismatch repair system is inactivated by oxidative stress (*33*). The antioxidant effect of glycyrrhizic acid in mice (*34*) suggests that suppression of oxidative stress by glycyrrhizic acid could be one of the mechanisms that resulted in the activation of repair enzymes much earlier than with UV radiation alone; thus it is possible that thymine dimers were removed much earlier in glycyrrhizic acid-treated animals than 1 h, the time we used in our study. More studies are needed employing different times to assess whether glycyrrhizic acid causes a faster repair of UV-radiation-induced DNA damage, ultimately leading to a strong reduction in thymine dimer-positive cells. Other possibilities could be that glycyrrhizic acid protects epidermal cells from UV-radiation-induced thymine dimer-positive cells by modulating DNA repair enzymes other than MSH2 and/or by an alteration of the ATM/ATR pathways. It is not known whether the effect of glycyrrhizic acid on these pathways is an upstream response for its efficacy against UV-radiation-induced thymine dimer-positive cells in epidermis.

p53 plays an important role in growth arrest and apoptosis. In response to DNA damage by UV radiation, p53 is upregulated to arrest the cell cycle through transcriptional activation of p21/Cip1 to facilitate DNA repair when the damage is mild or to induce apoptosis via activating apoptotic proteins such as Fas/Apo-1, Bax and DR5 or downregulating anti-apoptotic proteins such as cellular inhibitor of apoptosis protein 2 and bcl-2 when the damage is severe (*35, 36*). In the present study, we found that glycyrrhizic acid upregulated p53 with a concomitant increase in p21/Cip1 protein levels and decrease in apoptotic sunburn cells in UVB-irradiated skin. We further observed that UVB-radiation-induced PCNA-positive cells were inhibited by glycyrrhizic acid treatment. These findings establish a relationship between the formation of sunburn cells and apoptosis-related biomarkers. These results are in accord with the notion that inhibition of cell proliferation could be one of the mechanisms by which glycyrrhizic acid protects damaged cells from entering the cell cycle, thereby giving damaged cells sufficient time for repair and preventing their entry into an apoptotic pathway in case the damage is severe. Collectively, the proposed mechanisms for the protective effect of glycyrrhizic acid on UV-radiation-induced damage in epidermal cells are as follows: glycyrrhizic acid protects SKH-1 mouse epidermis from the DNA-damaging effects of UV radiation such as thymine dimer-positive cells, thereby decreasing UV-radiation-induced

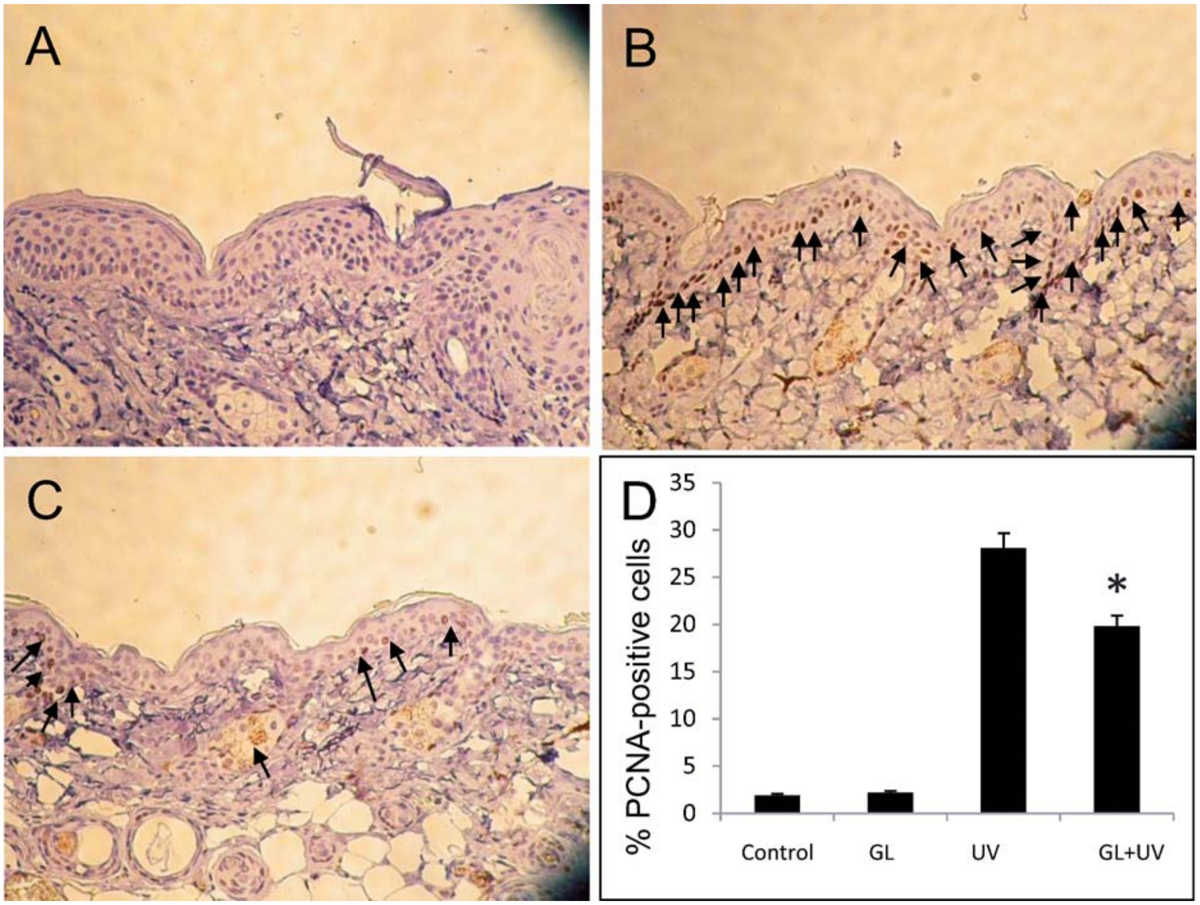

FIG. 6. Inhibition of UVB-radiation-induced PCNA-positive cells by glycyrrhizic acid (GL). Panel A, control; panel B, UV radiation; panel C, glycyrrhizic acid + UV radiation. Panel D shows the quantitative results for PCNA-positive cells. $*P < 0.01$.

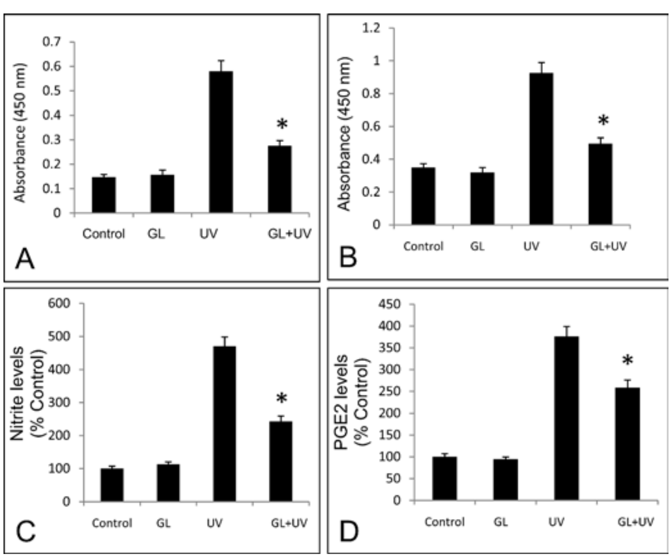

FIG. 7. Glycyrrhizic acid (GL) inhibits UVB-radiation-induced activation of NF-κB, COX-2, NO and PGE2 expression. Panel A, NF-κB; panel B, COX-2; panel C, NO; panel D, PGE2. Activities were calculated as means ± SE ($n = 5$). $*P < 0.003$ for NF-κB, $*P < 0.004$ for COX-2, $*P < 0.003$ for NO, and $*P < 0.01$ for PGE2.

apoptotic/sunburn cells. Further, glycyrrhizic acid inhibits UV-radiation-induced epidermal cell proliferation by decreasing PCNA, possibly through activation of p53-p21/Cip1, suggesting a cell growth delay rather than acceleration of cell death.

Exposure to UV radiation also results in the formation of reactive oxygen species and prostaglandins (*37*). UV-radiation-induced prostaglandins may play important roles in inflammation, photoaging and photocarcinogenesis in human skin. NO and prostaglandins, which are produced by iNOS and COX-2, respectively, have been implicated as important mediators in the processes of inflammation (*38*). Thus potential inhibitors of iNOS and COX-2 have been considered to be effective therapeutically for preventing inflammatory reaction and disease. Our results in this study clearly demonstrated that glycyrrhizic acid significantly suppressed UV-radiation-induced NO, COX-2 and PGE2 levels in mouse skin. Control of COX-2 induction involves a complex array of regulatory factors, including NF-κB (*39*). We found that feeding with glycyrrhizic acid was effective in terms of inhibiting NF-κB DNA binding. The inhibitory effect of glycyrrhizic acid on NF-κB activation by UVB radiation could be due to inhibition of IκB degradation and p65 translocation to the nucleus (*11*).

In this study, we used a single large dose (180 mJ/cm^2) of UVB radiation given to SKH-1 hairless mice, which is approximately six times higher than the physiologically relevant dose (30 mJ/cm^2/day). One reason for selecting a single large dose of UVB radiation is that it results in less stress on the animals. We also believe that a single large dose is well justified scientifically because outdoor occupational exposure of humans or a sunbathing exposure in the summer was reported to be 50–100 mJ/cm^2 per day (*40*). We hypothesized that if glycyrrhizic acid treatment can protect animals from UVB radiation after a single large dose, it is reasonable to expect that it could protect against fractionated, protracted irradiation.

Studies involving the simulation of solar optical radiation on a laboratory scale have taken advantage of the characteristics of xenon arc emission. Since the dose of UV radiation used in the standardized model is sufficient to cause tumors in all mice, the results of this study implicate UVB radiation as the main carcinogen for the tumors observed in this study.

In conclusion, our data clearly indicate that oral glycyrrhizic acid inhibits UVB-radiation-induced carcinogenesis and decreases several radiation-induced biomarkers. The molecular events associated with protective effect of glycyrrhizic acid in UVB-radiation-induced skin cancer are complex. They include downregulation of cell proliferative controls, alterations of thymine dimers, PCNA, apoptosis and NF-κB. IN addition, inflammatory responses involving COX-2, PGE2 and NO are involved, as well as the upregulation of p53 and p21/Cip1 to facilitate DNA repair. The efficacy of glycyrrhizic acid observed in the present study in terms of a decrease in tumor numbers and shrinkage of tumor size could have clinical significance. Therefore, the present study provides fundamental information on the effects of glycyrrhizic acid on mechanistically important early biomarkers for UVB-radiation-induced effects *in vivo*, suggesting a short-term model for evaluation of potential protective pharmacological modulators against UVB-radiation-induced damages. More mechanistic studies are needed to further clarify the effect of glycyrrhizic acid on UV-radiation-induced damages and their biological significance in both overall efficacy and safety of glycyrrhizic acid against photocarcinogenesis. Our results provide a focus for the rational development of glycyrrhizic acid as a safe and effective chemopreventive agent against UV-radiation-induced photoaging and photocarcinogenesis.

ACKNOWLEDGMENTS

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行政院國家科學委員會補助國內專家學者出席國際學術會議報告

報告人姓名：程兆明
服務機構及職稱：
中山醫學大學醫學系教授
會議時間、地點：100 年 4 月 2-6 日，美國佛羅里達州奧蘭多
會議名稱：第 102 次美國癌症學會年會
102st Annual Meeting of American Association for Cancer Research, 2011
發表論文題目：Molecular mechanisms underlying chemopreventive activities of glycyrrhizic acid against UVB-induced carcinogenesis in SKH-1 hairless mouse epidermis

一、參加會議經過：

這次會議仍然按往例分成十大主題進行口頭及壁報式發表論文：(1) Cellular and Molecular Biology (2) Tumor Biology (3) Carcinogensis (4) Chemistry (5) Clinical Research (6) Endocrinology (7) Epidemiology (8) Experimental and Molecular Therapeutics (9) Immunology (10) Prevention Research

大會有專題演講、論壇、座談會、討論、訓練課程、及研究特殊成就之專題報告：(1) Symposia (2) Public Forum (3)Professional Advancement Series (4) Educational Sessions (5) Methods Workshops (6) Associate Member Council Sessions (7) Awards and Lectureships (8) Chemistry in Cancer Research Working Group Sessions (9) Late-Breaking Research Sessions (10) Meet-the-Expert Sunrise Sessions (11) Molecular Epidemiology Group Sessions (12) NCI/NIH Sessions (13) New Concepts in Organ Site Research (14) Plenary Sessions (15) Special Sessions (16) Town Meeings

會議由早上 7：00am 到下午 5:30pm 分別在不同會議室同時進行。

二、與會心得

這次會議的最大收穫還是聆聽尚未正式發表的第一手資料、新的發現及技術等等，同時再度與世界各國之專家學者面對面討論彼此合作之可能性。會議期間仍然依往例撥空到各個生技公司與儀器廠商展示會場參訪，並收集一些最新科技與資訊之資料。

四、攜回資料名稱及內容

會議論文摘要、光碟與會議內容目錄，新的實驗方法、新的試劑及新儀器簡介資料等。

AACR American Association for Cancer Research 102nd ANNUAL MEETING
April 2-6, 2011 • Orange County Convention Center, Orlando, Florida

January 25, 2011

Re: AACR 102nd Annual Meeting 2011 in Orlando
Temporary Abstract Number: 2035
Title: Molecular mechanisms underlying chemopreventive activities of glycyrrhizic acid against UVB-induced carcinogenesis in SKH-1 hairless mouse epidermis

Dear Dr. Cherng:

Your above-referenced abstract has been scheduled for presentation in a Poster Session at the 2011 AACR Annual Meeting in Orlando, FL and will be published in the 2011 Proceedings of the American Association for Cancer Research. Presentation information pertaining to your abstract is below:

Session ID: Tumor Biology 24
Session Date and Time: Monday Apr 4, 2011 1:00 PM - 5:00 PM
Location: Exhibit Hall A4-C, Poster Section

Permanent Abstract Number: 2483

Please refer to the printed Final Program [distributed onsite] or the online Annual Meeting Itinerary Planner [available in late February through the AACR Website at http://www.aacr.org/ for the exact location of your presentation.

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Thank you for your participation in the 2011 AACR Annual Meeting.

Sincerely,
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Molecular mechanisms underlying chemopreventive activities of glycyrrhizic acid against UVB-induced carcinogenesis in SKH-1 hairless mouse epidermis

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Abstract

Glycyrrhizic acid (GL) has been shown to possess anti-inflammation, antiviral, and chemoprotective activity against tumors. We assessed the protective effects of GL in UVB-induced skin tumor formation in SKH-1 hairless mice and the early molecular biomarkers. Mice irradiated at 180 mJ/cm^2 twice per week elicited 100% tumor incidence in 20 weeks. Feeding of GL prior to UVB irradiation caused a strong protection against photocarcinogenesis in terms of delay in tumor appearance, multiplicity, and size. It took 24 weeks to get 100% tumor incidence in GL plus UVB treated-group; the first tumor appearance in UVB-treated animals occurred at 16th week, which was delayed by 3 weeks in GL plus UVB-treated group. Feeding of GL for 2 weeks before a single UVB (180mJ/cm^2) irradiation resulted in a strong and significant decrease in UVB-induced thymine dimer-positive cells (p < 0.004), proliferative cell nuclear antigen PCNA (p < 0.01), terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, and apoptotic sunburn cells (p < 0.01) together with an increase in p53 and p21/Cip1-positive cell population in epidermis (p < 0.02 ~ 0.003). Simultaneously, GL also significantly inhibited NF-κB, cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), and nitric oxide (NO) levels (p < 0.01-0.004). Taken together, these results suggest that GL causes a strong protective effect against photocarcinogenesis via down-regulation of cell proliferative controls, involving thymine dimer, PCNA, apoptosis, transcription factors NF-kB and of inflammatory responses involving COX-2, PGE2, and NO, while up-regulation of p53, p21/Cip1 to prevent DNA damage. These results provide a focus for the rational development of GL as a novel chemopreventive agent against UV-induced human skin cancer.

Key words: Ultraviolet, Skin tumors, Glycyrrhizic acid, chemoprevention

國科會補助計畫衍生研發成果推廣資料表

日期:2011/07/26

國科會補助計畫	計畫名稱: 甘草酸與其衍生物對保護紫外線照射引起的皮膚老化與皮膚癌的分子機制
	計畫主持人: 程兆明
	計畫編號: 97-2320-B-040-033-MY3　　學門領域: 藥理及毒理

無研發成果推廣資料

97 年度專題研究計畫研究成果彙整表

計畫主持人：程兆明			計畫編號：97-2320-B-040-033-MY3				
計畫名稱：甘草酸與其衍生物對保護紫外線照射引起的皮膚老化與皮膚癌的分子機制							
成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	5	5	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	3	4	100%	篇	成果列為該期刊(Radiation Research)之封面
		研究報告/技術報告	0	0	100%		
		研討會論文	3	3	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	獲得獎項:免疫及風濕病醫學會年會海報論文獎 重要國際合作：與美國疾病管制中心資深研究員合作

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

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發現甘草酸及幾種天然成分對抗 UVB 很有效果，因此可供業界未來製成保護製劑。