Short Communication

Dietary Feeding of Silibinin Prevents Early Biomarkers of UVB Radiation–Induced Carcinogenesis in SKH-1 Hairless Mouse Epidermis

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Abstract

Solar radiation is the causal etiologic factor in the development of nonmelanoma skin cancer (NMSC). Depletion of the stratospheric ozone layer leads to an increase in ambient UV radiation loads, which are expected to further raise skin cancer incidence in many temperate parts of the world, including the United States, suggesting that skin cancer chemopreventive approaches via biomarker efficacy studies or vice versa are highly warranted. Based on our recent study reporting strong efficacy of silibinin against photocarcinogenesis, we assessed here the protective effects of its dietary feeding on UVB-induced biomarkers involved in NMSC providing a mechanistic rationale for an early-on silibinin efficacy in skin cancer prevention. Dietary feeding of silibinin at 1% dose (w/w) to SKH-1 hairless mice for 2 weeks before a single UVB irradiation at 180 mJ/cm² dose resulted in a strong and significant \( P < 0.001 \) decrease in UVB-induced thymine dimer–positive cells and proliferating cell nuclear antigen, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling, and apoptotic sunburn cells together with an increase \( P < 0.001 \) in p53 and p21/cip1-positive cell population in epidermis. These findings suggest that dietary feeding of silibinin affords strong protection against UVB-induced damages in skin epidermis by (a) either preventing DNA damage or enhancing repair, (b) reducing UVB-induced hyperproliferative response, and (c) inhibiting UVB-caused apoptosis and sunburn cell formation, possibly via silibinin-caused up-regulation of p53 and p21/cip1 as major UVB-damage control sensors. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1344–9)

Introduction

The incidence of skin cancer has been increasing over the past several decades, and it is estimated that over 1 million new cases of nonmelanoma skin cancer (NMSC) occur each year in the United States alone (1). Acute UV irradiation (a single exposure), such as thymine dimers and (6-4) photoproducts, induces DNA lesions that could lead to DNA mutations during replication if left unrepaired (2). The mutagenic and carcinogenic effects of UV radiation are attributed to the induction of DNA damage and errors in repair and replication, although exposed cells are equipped with a variety of mechanisms that constantly monitor and repair most of the damages inflicted by UV light (3). Nucleotide excision repair system prevents the DNA damages from leading to DNA mutations and subsequently skin carcinogenesis; in this process, p53 tumor suppressor gene plays a pivotal role by causing cell cycle arrest providing additional time for DNA repair or inducing cell death by apoptosis when the DNA damages are too severe to repair (2, 4). Recently, efforts to reveal the relationship between food intake and human health, including skin condition, have been increasing (5). It is well known that the appearance and functioning of skin is affected by the nutritional status where certain antioxidants are known to counteract and prevent UV-induced photodamage in skin epidermis (6). Dietary supplementation with vitamins, minerals, or essential fatty acids results in improved skin condition in animals; it has been reported that nutrients, such as vitamin A, E, C, and herbal extracts, including green tea, protect skin against photodamage (6, 7). In addition, use of diet/diet-derived agents as well as those used as dietary supplements, either topically or in diet as a chemopreventive agent, is considered as a less toxic and more effective approach in the chemoprevention of skin cancer compared with chemotherapeutic agents (8-10). Recently, we have reported that a naturally occurring flavonoid agent, silibinin, which is consumed widely by humans around the world including the United States as dietary supplement for its strong antihapatotoxic efficacy, has strong promise and potential in preventing (a) UVB-induced skin damages when applied topically (11) and (b) photocarcinogenesis when applied topically or fed in diet (12). We also showed dual efficacy of silibinin, as a UVB damage sensor, in protecting or enhancing apoptosis in HaCaT cells depending on the extent of UVB-caused damage (13). Because silibinin is consumed p.o. as a supplement and because the p.o. route of agent delivery is possibly a more practical and translational approach, here we further extended our recently completed studies to assess protective effects of dietary feeding of silibinin on UVB-induced skin epidermal damages used as biomarkers in NMSC. Our results indicate that dietary feeding of silibinin would be effective in the management of acute solar injuries that trigger DNA damage, cell proliferation, and apoptosis, and provide a mechanistic rationale for an early-on silibinin efficacy in skin cancer prevention.
Materials and Methods

Chemicals and Animals. Silibinin was obtained from Sigma, Co. (St. Louis, MO). Silibinin purity was analyzed as a pure agent by high-performance liquid chromatography as reported earlier (14), and 1% (w/w) silibinin diet was prepared commercially by Dyets, Inc. (Bethlehem, PA). Female SKH-1 hairless mice (5 weeks old) were obtained from Charles River Laboratories (Wilmington, MA) and maintained in animal house facility at the University of Colorado Health Sciences Center under standard laboratory condition (temperature 24 ± 2°C, relative humidity 50 ± 10%, 12 hours light/12 hours dark cycle). Animals were fed control or silibinin test diet and water ad libitum.

Exposure of Mice to UVB Radiation. The UVB light source was a bank of four FS-40-T-12-UVB sunlamps equipped with a UVB Spectra 305 Dosimeter (Davalin, Co., Bryan, OH), which emitted ~80% radiation in the range of 280 to 340 nm with a peak emission at 314 nm as monitored with a SEL 240 photodetector, 103 filter, and 1008 diffuser attached to an IL1400A Research Radiometer (International Light, Newburyport, MA). The UVB irradiation dose was also calibrated using IL1400A radiometer. Mice were exposed to UVB irradiation as reported earlier (11).

Experimental Design. A short-term study was designed to assess the protective effects of dietary silibinin on acute (single exposure, 180 mJ/cm²) UVB irradiation-induced skin damage and associated molecular events in SKH-1 hairless mice. Animals were divided into four groups containing five animals in each group for each time point of the study as follows: (a) unexposed and untreated control mice (control), (b) animals fed with 1% (w/w) silibinin in diet for 2 weeks (SBP), (c) animals irradiated once with 180 mJ/cm² UVB dose, and (d) animals fed with 1% (w/w) silibinin in diet for 2 weeks and irradiated with same dose of UVB (SBF + UVB). The dietary silibinin dose selection in the present study is based on our recently published report where 1% (w/w) long-term silibinin dietary feeding has been found safe and nontoxic with potent cancer preventive efficacy against photocarcinogenesis in SKH-1 hairless mouse model (12). Animals were sacrificed after 1 and 8 hours of UVB exposure and dorsal skin was collected. Samples from 1 hour were used for the analysis of thymine dimer–positive cells and 8-hour samples were used for the analysis of p53, p21/Cip1, proliferating cell nuclear antigen (PCNA), deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL), and apoptotic sunburn cells. The selection of these two time points for the molecules was based on our recently published report where 1% (w/w) long-term silibinin dietary feeding has been found safe and nontoxic with potent cancer preventive efficacy against photocarcinogenesis in SKH-1 hairless mouse model (12). Animals were sacrificed after 1 and 8 hours of UVB exposure and dorsal skin was collected. Samples from 1 hour were used for the analysis of thymine dimer–positive cells and 8-hour samples were used for the analysis of p53, p21/Cip1, proliferating cell nuclear antigen (PCNA), deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL), and apoptotic sunburn cells. The selection of these two time points for the molecules to be analyzed was based on a recent study showing their maximum induction in skin epidermis following a single UVB exposure (at 180 mJ/cm² dose) of SKH-1 mice (11).

Immunohistochemical Analysis. Following desired treatments and/or UVB irradiation, dorsal skin was collected, fixed in 10% phosphate-buffered formalin for ~10 hours at 4°C, dehydrated in ascending concentration of ethanol, cleared in xylene, and embedded in PolyFin (Triangle Biomedical Sciences, Durham, NC). Four-micrometer serial sections were cut and processed for immunohistochemical staining. In all the immunohistochemical staining, to rule out the nonspecific staining allowing better interpretation of specific staining at the antigenic site, negative staining controls were used in which sections were incubated with N-Universal Negative Control mouse or rabbit antibody (DAKO, Carpinteria, CA) under identical conditions as desired. All the microscopic immunohistochemical analyses were done using Zeiss Axioscop 2 microscope (Carl Zeiss, Inc., Jena, Germany). Pictures were taken by Kodak DC290 camera under ×400 magnification and processed by Kodak Microscopy Documentation System 290 (Eastman Kodak Company, Rochester, NY).

Immunostaining for Thymine Dimer–Positive Cells. Briefly, endogenous peroxidase activity was blocked by 10-minute incubation with 3% hydrogen peroxide, and slides were then incubated with 0.125% trypsin for 10 minutes at 37°C and then with 1 N HCl for 30 minutes at room temperature. The sections were incubated with peroxidase-conjugated monoclonal antithymine dimer IgG1 antibody (Kamiya Biomedical, Co., Seattle, WA) for 90 minutes at room temperature. Color was developed by incubating the sections in 3,3-diaminobenzidine for 10 minutes at room temperature. The sections were counterstained with hematoxylin, dehydrated, and mounted. Sections were examined and the number of nuclei that displayed positive (brown) staining was determined through-out the entire available epidermis. Data is presented as percent of total cells counted.

Immunostaining for p53. The tissue sections were deparaffinized, rehydrated, and treated with 10 mmol/L sodium citrate buffer (pH 6.0) in a microwave for 5 minutes at full power for antigen retrieval. The sections were then quenched of endogenous peroxidase activity by immersing in 5% hydrogen peroxide for 5 minutes at room temperature. The sections were incubated with anti-p53 antibody (Novocastra Laboratories, Ltd., Newcastle-upon-Tyne, United Kingdom) at 1:200 dilution in PBS for 2 hours at room temperature in humidity chamber followed by overnight incubation at 4°C. The sections were then incubated with biotinylated anti-rabbit secondary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 45 minutes at room temperature followed by 45-minute incubation with conjugated horseradish peroxidase streptavidin. Color development was achieved by incubation with 3,3-diaminobenzidine for 10 minutes at room temperature. The sections were counterstained with hematoxylin, dehydrated, and mounted.

Immunostaining for p21/cip1. The tissue sections were deparaffinized, rehydrated, and treated with 10 mmol/L sodium citrate buffer (pH 6.0) in a microwave for 5 minutes at full power for antigen retrieval. The sections were then quenched of endogenous peroxidase activity by immersing in 5% hydrogen peroxide for 5 minutes at room temperature. The sections were then incubated with anti-p21/cip1 antibody (mouse anti-p21 monoclonal antibody, BD Biosciences, San Diego, CA) at 1:100 dilution in PBS for 2 hours at room temperature in humidity chamber. The sections were then incubated with biotinylated rabbit anti-mouse antibody IgG (DAKO) at 1:300 dilution for 1 hour at room temperature. Thereafter, sections were incubated with conjugated horseradish peroxidase streptavidin (DAKO) at 1:500 dilution in PBS for 30 minutes at room temperature in humidity chamber. The sections were then incubated with 3,3-diaminobenzidine working solution for 5 to 10 minutes at room temperature and counterstained with diluted hematoxylin for 2 minutes and rinsed in Scott’s water. The slides were then dehydrated and mounted.

Immunostaining for PCNA. The paraffin-embedded sections were heat immobilized and deparaffinized using xylene and rehydrated in a graded series of ethanol with a final wash in distilled water. Antigen retrieval was done in 10 mmol/L citrate buffer (pH 6.0) in microwave for 2 and 18 minutes at full and 20% of power levels, respectively. Endogenous peroxidase activity was blocked by immersing the sections in 3.0% H₂O₂ in methanol (v/v), and the sections were then incubated with mouse monoclonal anti-PCNA antibody (DAKO) at 1:200 dilution in PBS for 2 hours at 37°C in humidity chamber. The sections were then incubated with biotinylated rabbit anti-mouse antibody IgG (DAKO) at 1:300 dilution for 1 hour followed by incubation with conjugated horseradish peroxidase streptavidin (DAKO) at 1:500 dilution in PBS for 30 minutes at room temperature in humidity chamber. Sections were then incubated with 3,3-diaminobenzidine working solution for 5 to 10 minutes at room temperature and counterstained with diluted hematoxylin for 2 minutes and rinsed in Scott’s water. The slides were then dehydrated and mounted.

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solution for 10 minutes at room temperature and counterstained with diluted hematoxylin for 2 minutes and rinsed in Scott's water. Finally, sections were dehydrated and mounted for microscopic observation.

Measurement of Apoptotic Sunburn Cells. Apoptotic cells are morphologically distinct due to cell shrinkage and nuclear condensation, which attributes to their small, dense nuclei and eosinophilic cytoplasm that stain darker by H&E. Based on these criteria, sections were stained with H&E and apoptotic sunburn cells were counted.

TUNEL Staining for Apoptotic Cells. Apoptotic cells were detected using the DeadEnd Colorimetric TUNEL system (Promega, Madison, WI) following manufacturer's protocol with some modifications. In brief, tissue sections after deparaffinization and rehydration were permeablized with proteinase K (30 μg/mL) for 1 hour at 37°C. Thereafter, the sections were quenched of endogenous peroxidase activity using 3% hydrogen peroxide for 10 minutes. After thorough washing with 1× PBS, sections were incubated with equilibration buffer for 10 minutes and then terminal deoxynucleotidyl transferase reaction mixture was added to the sections, except for the negative control, and incubated at 37°C for 1 hour. The reaction was stopped by immersing the sections in 2× SSC buffer (Sigma) for 15 minutes. Sections were then treated with conjugated horseradish peroxidase streptavidin (1:500) for 30 minutes at room temperature, and after repeated washing, sections were incubated with 3,3′-diaminobenzidine for ~10 minutes for color development. Sections were mounted after dehydration and observed under ×400 magnification for TUNEL-positive cells.

Statistical Analysis. Data were analyzed using the SigmaStat 2.03 software. The statistical significance of difference between UVB alone versus all other groups was determined by one-way ANOVA followed by Bonferroni t-test for multiple comparisons. P < 0.05 was considered statistically significant.

Results

Dietary Feeding of Silibinin Reduces UVB-Induced Thymine Dimer–Positive Cells in Mouse Skin. Thymine dimers are formed in DNA immediately after UVB irradiation and are considered as an early and important biomarker for UVB-induced DNA damage. Therefore, thymine dimers are also used as a biomarker to study the protective effect of agents against UVB photodamage (4). Accordingly, we first assessed the effect of dietary feeding of silibinin (1% w/w, for 2 weeks) on thymine dimer–positive cells in mouse epidermis 1 hour after UVB exposure, a time point that showed maximum number of thymine dimer–positive cells (4, 11). As shown in Fig. 1A-C, compared with sham irradiated controls, a single exposure of mice to UVB (180 mJ/cm²) strongly induced the formation of thymine dimer–positive cells; however, dietary feeding of silibinin before UVB exposure resulted in a strongly reduced thymine dimer–positive cell population. Quantitative analysis of immunostained skin tissue sections revealed that UVB exposure resulted in 51.0 ± 3.0% thymine dimer–positive cells in the epidermis, which was higher than the unexposed controls showing no positive cells or only silibinin-fed groups with 1.7 ± 0.2% positive cells (P < 0.001; Fig. 2A).

Dietary feeding of silibinin followed by a single UVB exposure, however, resulted in only 14.8 ± 2.1% thymine dimer–positive cells, accounting for 71% reduction (P < 0.001) compared with UVB alone group (Fig. 2A).

Dietary Feeding of Silibinin Further Up-Regulates UVB-Induced p53 and p21/cip1. In response to DNA damage, p53 and p21/cip1 are up-regulated for cell cycle arrest to facilitate DNA repair (4). In our study, feeding of silibinin followed by UVB exposure increased the number of p53-positive cells compared with UVB treatment alone (Fig. 1D-F). Exposure of SKH-1 mice to UVB resulted in 22.2 ± 2.0% p53-positive cells compared with 2.5 ± 0.3% and 4.1 ± 0.8% in unexposed control and silibinin feeding alone groups, respectively (Fig. 2B). Silibinin feeding followed by UVB radiation showed 29.3 ± 1.9% p53-positive cells, which was significantly higher (1.3-fold, P < 0.001) than that of UVB treatment alone (Fig. 2B). Similar to p53, strong immunostaining for p21/cip1 was also observed in UVB-exposed skin tissue sections, which increased further in silibinin-fed plus UVB-exposed group (data not shown). Quantification of the immunostaining data showed that UVB exposure resulted in 12.2 ± 1.7% p21/cip1-positive cells (P < 0.001), compared with 0.9 ± 0.4 and 1.1 ± 0.2 in unexposed control and silibinin alone groups, respectively (Fig. 2C). Silibinin feeding followed by UVB treatment showed 18.3 ± 1.3% p21/cip1-positive cells accounting for 1.5-fold increase (P < 0.001) compared with UVB treatment alone (Fig. 2C).

Dietary Feeding of Silibinin Inhibits UVB-Induced Epidermal Cell Proliferation. PCNA is an auxiliary protein of DNA polymerase-α and its high levels of expression correlate cell proliferation, suggesting that PCNA is an excellent marker of cellular proliferation, which also serves as an effective prognostic indicator of initiated cancer cells (15, 16). As shown in Fig. 1G-I, a single UVB irradiation (180 mJ/cm²) of mice resulted in a strong PCNA-positive staining in skin epidermis compared with unexposed control and silibinin-fed groups; however, it was considerably reduced in the group fed with silibinin before UVB exposure. Quantitative analysis of the PCNA immunostaining showed that UVB irradiation caused 20.2 ± 1.5% PCNA-positive cells compared with 3.7 ± 0.9 and 2.4 ± 0.5% in unexposed control and silibinin alone groups, respectively (Fig. 2D). When mice were fed with silibinin before UVB exposure, only 12.9 ± 1.3% PCNA-positive cells were observed, which accounted for 34% (P < 0.001) inhibition in cell proliferation compared with UVB alone group (Fig. 2D).

Dietary Feeding of Silibinin Inhibits Formation of Sunburn Cells and Apoptosis. Consistent with above results, silibinin feeding also significantly inhibited UVB-induced sunburn cells and apoptosis. Characteristic dyskeratotic sunburn cells with pyknotic nuclei were detected by histomorphologic analysis by H&E staining and further confirmed by TUNEL assay. UVB irradiation induced the formation of apoptotic sunburn cells and a noticeable protective effect was observed in mice fed with silibinin (43% reduction, P < 0.001) before UVB irradiation (Fig. 2E). UVB irradiation increased the number of apoptotic cells to 18.9 ± 2.4% as observed by TUNEL staining (Fig. 1J-L) compared with 25.5 ± 0.7% and 4.1 ± 0.5% in unexposed and silibinin fed groups, respectively (Fig. 2F). Dietary feeding of silibinin followed by UVB exposure resulted in 10.2 ± 1.2% TUNEL-positive cells, which accounted for 46% decrease (P < 0.001) when compared with the UVB alone group (Fig. 2F). These results suggest the strong protective effect of silibinin on UVB-induced sunburn cell formation and apoptosis in mouse skin.

Discussion

The primary function of epidermis is to provide a protective barrier against numerous environmental insults, including UV radiation. UVB is a potent carcinogen known to damage DNA directly or through the generation of free radicals. Protective measures, such as apoptosis and inflammation, are suggested to be beneficial in safeguarding skin epidermis in long term against the propagation of potentially tumorigenic cells generated after a high dose of UV irradiation (17). However,
these biological events may be acutely detrimental to the architectural and functional integrity of the tissue owing to rampant cell death and inflammatory responses, leading to epidermal erosion and consequently loss of barrier functions (17, 18). These studies suggest that protecting skin against UVB-induced biological responses would be effective in reducing skin damage as well as NMSC. With this rationale, in the present study, we evaluated the protective efficacy of dietary feeding of silibinin against UVB-induced skin damage. Our results show a strong suppression of UVB-induced damage by dietary feeding of silibinin via an inhibition of DNA damage (and/or induction in repair), cell proliferation, and apoptosis, together with an induction of p53 and p21/cip1 in epidermal cells.

Silibinin is a nontoxic phytochemical even at very high doses and there is no known LD$_{50}$ for silibinin as observed from animal studies (19). We have used higher doses of silibinin, up to 2 g/kg/d by p.o. gavage or 1% (w/w) in diet, in different in vivo mouse models that did not show any untoward health effects, including diet consumption and body weight gain (12, 14, 20). Silibinin is also used as hepatoprotective drug and dietary supplement without any considerable untoward health effects in humans (19, 21). In addition to the findings in the present study, various studies by us have shown chemopreventive efficacy of silibinin in in vitro (13, 22) as well as in vivo animal skin cancer models, suggesting that silibinin could be a potential agent for chemopreventive drug development against skin cancer (11, 12).

Thymine dimers are formed immediately on DNA following absorption of UVB energy and, therefore, represent an early biomarker of UVB-induced DNA damage (4). In the present study, we observed that dietary feeding of silibinin strongly reduced the formation of UVB-induced thymine dimer–positive cells in the mouse skin epidermis. In a recently completed study, we observed a similar inhibitory effect of topical application of silibinin on UVB-induced thymine dimer

![Figure 1](image-url)

**Figure 1.** Effect of dietary feeding of silibinin on UVB-induced thymine dimer, p53, PCNA, and TUNEL-positive cells in mouse skin as assessed by immunohistochemical analysis. SKH-1 hairless mice were unexposed (control), fed with 1% silibinin diet (w/w) for 2 weeks (SBF), exposed with UVB (180 ml/cm$^2$) alone once (UVB), and fed with 1% silibinin diet (w/w) for 2 weeks followed by a single UVB exposure (SBF + UVB) as detailed in Materials and Methods. Immunohistochemical staining for thymine dimer (A–C), p53 (D–F), PCNA (G–I), and TUNEL (J–L) was done as described in Materials and Methods. In each case, only representative data are shown for different treatments as labeled in the figure. Pictures are shown at $\times 400$ magnification.
formation, which possibly had both sunscreen effect and an interaction of silibinin at molecular level in skin epidermis (11). In this scenario, the findings in the present study provide a stronger evidence for the latter mechanism, without any involvement of sunscreen effect, via physiologic distribution of dietary-fed silibinin in skin, which is supported by the fact that dietary fed silibinin/silymarin is physiologically available in skin (14).

The tumor suppressor p53 plays a crucial role in the protection against DNA damage allowing cell cycle arrest, DNA repair, or apoptosis by transcriptional activation of p53-related genes, such as p21/cip1 and Bax (23). Consistent with this report, we observed that dietary feeding of silibinin resulted in an enhancement of UVB-induced p53 as well as p21/cip1 protein expression. These proteins are generally up-regulated in response to UVB-caused DNA damage leading to cell cycle arrest to allow enough time for DNA repair and avoid replication error (4). This observation is consistent with previous studies in the same animal model showing stimulatory effect of chemopreventive agents, such as green tea or caffeine and silibinin, on the protein levels of p53 and downstream effector p21/cip1 (11, 24). Further, it seems likely that up-regulation of p21/cip1 could have been mediated via p53 by silibinin, as p21/cip1 is a p53 responsive gene. However, more studies are needed in the future to further support this anticipation.

PCNA is implicated in DNA replication and repair by forming a sliding platform that could mediate the interaction of numerous proteins with DNA (16). It is known that p21/cip1 binds to PCNA and inhibits PCNA function in DNA replication (25). Another study has shown that phosphorylation of specific residues within the PCNA-binding motif can abrogate the p21/cip1-PCNA interaction, and that these residues are phosphorylated in vivo (26). Therefore, PCNA is regarded as an important target for p21/cip1 as well as a reliable biomarker for cell proliferation. Our results show that UVB-induced PCNA-positive cells were strongly inhibited by dietary feeding of silibinin. This suggests that inhibiting cell proliferation could be one of the mechanisms by which silibinin protects damaged cells from entering the cell cycle, thus affording these cells additional time for DNA damage repair to avoid replication error that otherwise often results in carcinogenic mutation. Cells with DNA damage, if left unrepaired, may follow the path of apoptotic cell death (24). By inhibition of cell cycle progression, allowing efficient DNA repair, the number of apoptotic or sunburn cells will more likely be reduced with silibinin treatment. This is in accord with our results where silibinin decreased the number of UVB-induced TUNEL-positive as well as apoptotic sunburn cells in skin epidermis. Overall, these protective effects of silibinin are supposed to inhibit UVB-induced skin carcinogenesis as we observed in a recently completed study (12).

In summary, our data show that dietary feeding of silibinin to SKH-1 mouse for 2 weeks before irradiation with a single dose of UVB enhances UVB-induced p53 and p21/cip1 as a possible mechanism to protect skin epidermal cells from DNA damage and inhibit proliferation and apoptosis or sunburn cell formation. Future studies are needed to further decipher the involvement of these molecular and biological events in cell cycle arrest and DNA damage repair by silibinin in its overall chemopreventive efficacy against photocarcinogenesis.

Figure 2. Effect of dietary feeding of silibinin on UVB-induced thymine dimer, p53, p21/Cip1, PCNA, apoptotic sunburn, and TUNEL-positive cells in mouse skin epidermis as assessed by quantification of immunohistochemically stained tissue sections. SKH-1 hairless mice were unexposed (control), fed with 1% silibinin diet (w/w) for 2 weeks (SBF), exposed with UVB (180 mJ/cm²) alone once (UVB), and fed with 1% silibinin diet (w/w) for 2 weeks followed by a single UVB exposure (SBF + UVB) as detailed in Materials and Methods. A, Percent of thymine dimer–positive cells. B, Percent of p53-positive cells. C, Percent of p21/Cip1-positive cells. D, Percent of PCNA-positive cells. E, Percent of sunburn cells from H&E staining. F, Percent of TUNEL-positive cells over total cells in randomly selected fields (>400) from each tissue sample. Columns, mean of 20 fields per five mice per group; bars, SE. The statistical significance of difference between UVB-treated and all other groups was determined by one-way ANOVA followed by Bonferroni t-test for multiple comparisons. a, P ≤ 0.001 compared with UVB.

Figure 2.
References

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