Differential Regulation of *P53* and *Bcl-2* Expression by Ultraviolet A and B

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The induction of apoptosis by ultraviolet (UV) radiation and other DNA damaging agents plays a critical role in monitoring the accumulation of genetic damage and the suppression of tumor development. We hypothesize that UVA and UVB induce apoptosis by modulating balances between *p53* and/or *bcl-2* genes. Using MCF-7 cells that express both wild-type *P53* and *Bcl-2* proteins, we demonstrated that UVA and UVB induced apoptosis through regulating expression of apoptosis promoting or inhibiting genes. UVA induced immediate apoptosis and downregulated *bcl-2* expression. *Bcl-2* expression was reduced by ≈40% at 4 h post-150 kJ UVA irradiation per m^2 with a maximum downregulation (over 70%) at 24 h. The dose–response studies revealed that significant reduction of *bcl-2* expression was observed at UVA doses ranging from 50 to 200 kJ per m^2; however, *p53* levels were not affected by UVA. In contrast, UVB exhibited a entirely different action than UVA in that UVB substantially induced *p53* expression, but had no effect on *bcl-2* expression. The induction of *P53* by UVB was dose and time dependent with the maximum expression at 24 h post-2 and post-4 kJ UVB irradiation per m^2. Downregulation of *bcl-2* and fragmentation of DNA induced by UVA occurred earlier (approximately at 4 h) than upregulation of *p53* and DNA fragmentation by UVB (12–24 h). These results suggest that UVA and UVB cause cell damage through different mechanisms and that the balances between the expression of *p53* and *bcl-2* may play an important role in regulating the apoptosis induced by UV irradiation. *Key words: apoptosis/p53 expression/ultraviolet radiation. J Invest Dematol 111:380–384, 1998*

Skin cancers are the most frequent human malignancies and are equal in their incidence to all other cancers combined in the U.S.A. (Scott *et al*, 1982). Epidemiologic, clinical, and biologic studies strongly suggest that UV irradiation from sunlight is an etiologic agent for the majority of human skin cancers (Elder, 1989). Because of the absorption of the atmosphere, short-wavelength UVC (200–290 nm) cannot reach the surface of the earth, whereas the medium-wavelength UVB (290–320 nm) and long-wavelength UVA (320–400 nm) constitute the major carcinogenic components of sunlight (Yuspa and Dlugosz, 1991; Cruijl *et al*, 1993). Thus, an understanding of the differential mechanisms of UVA- and UVB-induced biochemical events may contribute to the prevention of the UV-induced photodamage and skin cancers.

Apoptosis is a unique pathway of a programmed cell death upon developmental or environmental stimuli. Induction of apoptosis by ultraviolet and other DNA damaging agents plays a critical role in monitoring the accumulation of genetic damage and the suppression of tumor development (Lee and Bernstein, 1995). Numerous genes that encode products to regulate apoptosis have been identified, among which the tumor suppressor gene *p53* and apoptosis inhibitor gene *bcl-2* are considered to play important roles. *P53* is a transcription factor that has at least two functions (Kuerbitz *et al*, 1992; Lowe *et al*, 1993a): one is to act as a cell cycle checkpoint for arresting cells at the G1 to S transition, which enables the cells to repair any damages; the other function is to induce the unrepaired cells to undergo apoptosis, thus eliminating the cell as a potential source of persistent somatic mutation. Loss of function of the *p53* by either mutation or allele loss is a common finding in many human malignancies, including skin cancers (Romerdahl *et al*, 1989; Hollstein *et al*, 1991). It has been reported that human tumor cell lines containing wild-type *p53* were radiosensitive, whereas cell lines with mutated *p53* were radioresistant (McIlwrath *et al*, 1994). Cells from *p53* deficient mice were unable to undergo apoptosis induced by ionizing radiation (Clarke *et al*, 1993) or by chemotherapeutic drugs (Lowe *et al*, 1993b). So *p53* stands at the crossroads of tumor genesis and DNA damage.

Bcl-2, the B-cell lymphoma/leukemia-2, is an apoptosis inhibitor gene that prevents or reduces cell apoptosis induced by a wide range of stimuli, including UV radiation (Reed, 1994). It was reported that a single UV exposure caused a decline in *bcl-2* transcripts in rat skin (Gillardon *et al*, 1994) and that nerve growth factor protected melanocytic cells from UV-induced apoptosis by upregulating *bcl-2* expression (Zhai *et al*, 1996). These results suggest that the balance between the expression of *p53* and the expression of *bcl-2* is a central point for determining apoptosis to radiation (Chiarugi *et al*, 1995). Although the exact mechanism of action by which *bcl-2* functions is largely unknown, it is suggested that *bcl-2* blocks a final common pathway leading to apoptotic cell death (Chiou *et al*, 1994). Recent evidence indicates that oxidative stress may play a central role in the regulation of apoptosis (Buttke and Sandstrom, 1994) and that *Bcl-2* protein may function as an antioxidant, regulating levels of reactive oxygen species and controlling early entry into apoptosis (Hockenberry *et al*, 1993). It is known that the mechanisms of action of UVA and UVB on cells are significantly different. UVB mainly causes direct DNA damage such as pyrimidine dimers, whereas UVA induces oxidative stress, cell membrane damage, and certain types of DNA damage, mostly in the form of single-strand breaks and protein-DNA crosslinks (Godar *et al*, 1993). The differential action of UVA and UVB on apoptosis, however, is poorly understood. It was proposed that at

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the equal lethal dose, UVA caused immediate apoptosis (usually less than 4 h), predominantly initiated by membrane damage, whereas UVB induces delayed apoptosis (usually longer than 20 h), mainly initiated by DNA damage (Godar and Lucas, 1995). In this study, we have investigated the effects of UVA and UVB on apoptosis and the apoptosis-associated gene expression in MCF-7, a cell line expressing both wild-type *p53* and *bcl-2*. Our data showed that UVA induced immediate apoptosis primarily through downregulation of *bcl-2* expression, whereas UVB induced delayed apoptosis, characterized by induction of *p53* expression. These findings suggest that both UVA and UVB can induce apoptosis by triggering different pathways and that the balance between the expression of *p53* and the expression of *bcl-2* plays an important role in regulating UV-induced apoptosis.

MATERIALS AND METHODS

Chemicals and reagents Penicillin, streptomycin, and Hoechst 33258 were purchased from Sigma (St. Louis, MO). Double minimal Eagles medium and fetal bovine serum were purchased from GIBCO (Long Island, NY). *In situ* Apoptosis Kit was obtained from Boehringer Mannheim (Indianapolis, IN). Monoclonal antibodies against P53 protein (Ab-6), Bcl-2 protein (Ab-1), and tubulin (Ab-1) were purchased from Oncogene Science (Cambridge, MA). Horseradish peroxidase-conjugated goat anti-mouse IgG was obtained from Eappl (Durham, NC). Reagents for enhanced chemiluminescence were purchased from Amersham (Arlington Heights, IL).

Cell cultures The human breast cancer epithelial cell line MCF-7 (a gift from Dr. James Manfredi, Department of Neoplastic Disease, Mount Sinai Medical Center) was used in this study. This cell line is known to have the baseline expression of wild-type *P53* and *Bcl-2* proteins (Oncogene Science). The cells were incubated in double minimal Eagles medium supplemented with 10% bovine fetal calf serum, penicillin (75 units per ml), and streptomycin (25 mg per ml) at 37°C in a humidified 5% CO_2 atmosphere.

Measurement of cell survival Aliquots of 10^2, 10^3, or 10^4 cells were plated in triplicate in 25 cm^2 flasks. After attachment, cells were washed with phosphate-buffered saline (PBS) twice and then irradiated with appropriate doses of UVA or UVB. Cells were then incubated for 10 d, stained with Giemsa (GIBCO), and the number of colonies (grouping of 50 or more cells) counted. The survival fractions were determined relative to the sham control.

UV irradiation The cells were washed twice with PBS and then irradiated with appropriate UV doses. UVB irradiation was performed using two Westinghouse FS40 Sunlamps emitting a wavelength of 290–320 nm with a maximum peak at 313 nm (18 J per m^2 per s). UVA were produced by four Cosmolux A1–11 lamps emitting 320–400 nm with a maximum peak of 355 nm (28 J per m^2 per s). It has been reported that certain biologic responses such as components of MAP kinase transduction pathway could be activated by residual UVC portions of UVB lamps (Dhanwada *et al*, 1995). Therefore, polystyrene flask cover was used in the UVB study to filter out the wavelengths shorter than 290 nm and a Schott WG-335 filter was used in the UVA study to remove the wavelengths shorter than 335 nm. The UV fluences were monitored controlled by a Model IL1700 Research radiometer (International Light, Newburgport, MA) with a UVA probe (Model No. SED 038) and a UVB probe (Model No. SED 240).

Apoptosis assay The terminal deoxynucleotidetidyl transferase-mediated dUTP nicked-end labeling (TUNEL) was used to identified the apoptotic cells as described by Benassi *et al* (1997). The assay was performed with a slight modification as described in our previous publication (Brown *et al*, 1998). Briefly, cells were harvested by centrifugation at 1000 r.p.m. at 4°C for 10 min. The pellets were resuspended in 3.7% formaldehyde, incubated at the room temperature for 10 min, centrifuged for 10 min, and resuspended in 80% (vol/vol) ethanol at a concentration of 10^6 cell per ml. The cells were fixed onto gelatin-coated glass slides, air dried for 10 min, and dehydrated in 70% ethanol for 5 min. After drying for 2 h, the cells were treated with proteinase K (20 μg per ml) for 10 min and then with 2% hydrogen peroxide for 5 min. The cells were then labeled with a biotin-dUTP mixture using TdT kit. The slides were incubated at 37°C for 1 h, rinsed, and treated with strepavidin-horseradish peroxidase for 10 min. After rinsing with PBS, the slides were incubated with diaminobenzidine-H_2O_2 solution for 5 min, and then counter-stained with methyl green, dehydrated by successive washes with 95% ethanol, 100% ethanol, and xylene. Apoptotic cells were identified as the dark-brown stained cells and counted for 100 cells per slide under the microscopy.

DNA fragmentation assay Quantitation of fragmented DNA using a spectrophotofluorometer was performed as described Jarvis *et al* (1994) with a mild modification. Briefly, 3×10^5 cells were plated and incubated for 24 h prior to UV irradiation. Cells were harvested at different times after UV irradiation and centrifuged in 1.5 ml test tubes at 1500 r.p.m. for 10 min. Cell pellets were lyzed by addition of 0.2 ml lysing buffer (10 mM Tris, 1 mM ethylenediamine tetraacetic acid, 0.2% Triton X-100 at pH 7.5) for 30 min and then centrifuged at 13,000 r.p.m. for 20 min. Supernatant was transferred to a clean microcentrifuge tube. Aliquots of supernatant were admixed with Hoechst 33258 (1 μg per ml) in the buffer containing 154 mM NaCl and 15 mM Na_2 citrate (pH 7.0) and incubated at room temperature for 10 min in the dark. Fragmented DNA in cell supernatant was quantitated using a Fluorescence Spectrophotometer 650–10S (Perkin-Elmer, Watertown, MA) based upon the standard curve of highly purified calf thymus DNA as described by Jarvis *et al* (1994). The results were expressed as ng DNA per 3×10^5 cells minus the background from the untreated cells.

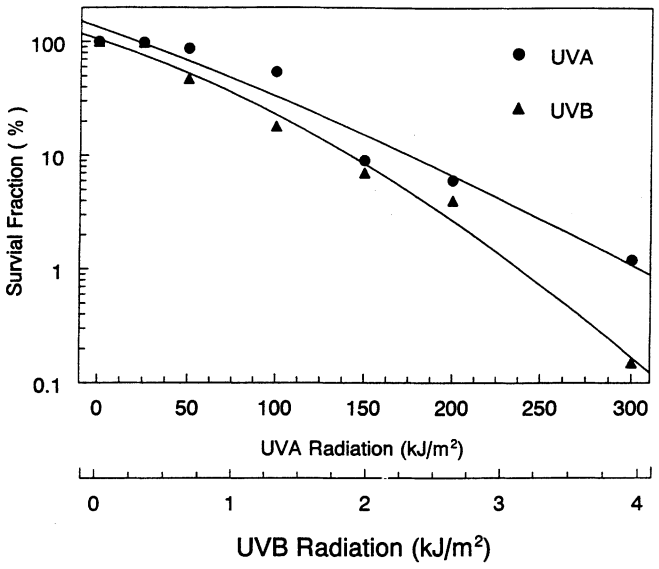

Figure 1. Survival curves for UV-irradiated cells. Each point represents the average values for three samples. The curves were fitted through a second-order regression analysis. The results are expressed as the survival fractions of cells after irradiation relative to the sham-irradiated controls.

Assays for *p53* and *bcl-2* expression Three million cells were plated for 24 h and then irradiated with different doses of UVA or UVB. After irradiation, cells were washed twice with cold PBS and harvested at different times in cold lysing buffer containing 50 mM Tris-HCl, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM ethylenediamine tetraacetic acid, 1 mM Na_3VO_4, 1 mM NaF, 10 ng leupeptin per ml, 20 ng of aprotinin per ml, and 1 mM phenylmethylsulfonyl fluoride. Cells were centrifuged at 4°C for 15 min and the supernatant was collected and normalized for protein concentration using BSA Protein Assay Kit (Pierce, Rockford, IL). Approximately 30 μg of protein from each sample were loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. After electrophoresis, separated proteins were transferred to nitrocellulose membranes and blotted in 5% nonfat dry milk overnight at 4°C. The membranes were then incubated with 2.5 μg monoclonal antibodies per ml against P53 or Bcl-2 proteins for 1 h at room temperature in 1% dry milk. After being washed three times with Tris-buffered saline, the membranes were incubated with 1:1000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG. The immunoreactivity was determined using the enhanced chemiluminescence. Equal protein loading was confirmed by hybridization with 1:1000 dilution of tubulin antibody.

RESULTS

Cell survival and morphologic changes upon UV irradiation The colony-forming assays were performed to evaluate the impact of UVA and B irradiation on the survival of MCF-7 cells. **Figure 1** shows the survival curves of cells exposed to different doses of UVA and UVB. From the survival curves, approximately equivalent cytotoxic doses of 150 kJ UVA per m^2 and 2 kJ UVB per m^2 were selected for the studies. After being exposed to 150 kJ UVA per m^2 or 2 kJ UVB per m^2, cells underwent the striking morphologic changes. **Figure 2** shows that at 48 h post-UVA and UVB irradiation, the majority of cells became rounded and detached from the culture dishes

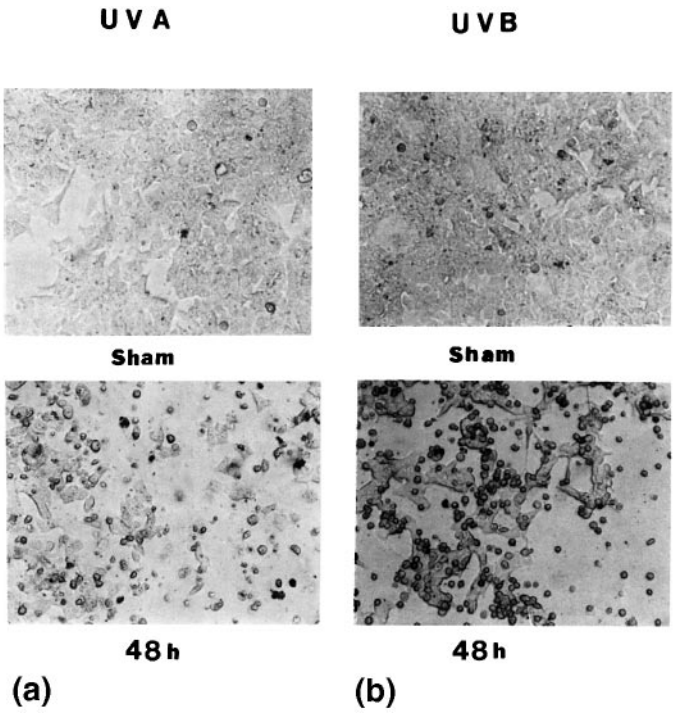

Figure 2. UVA- and UVB-induced morphologic alterations of MCF-7 cells. Cells were washed with PBS twice and followed by UV irradiation at appropriate doses. Upper traces are cells treated with sham irradiation and lower traces are cells at 48 h post-irradiation of 150 kJ UVA per m^2 (*a*) and 2 kJ UVB per m^2 (*b*).

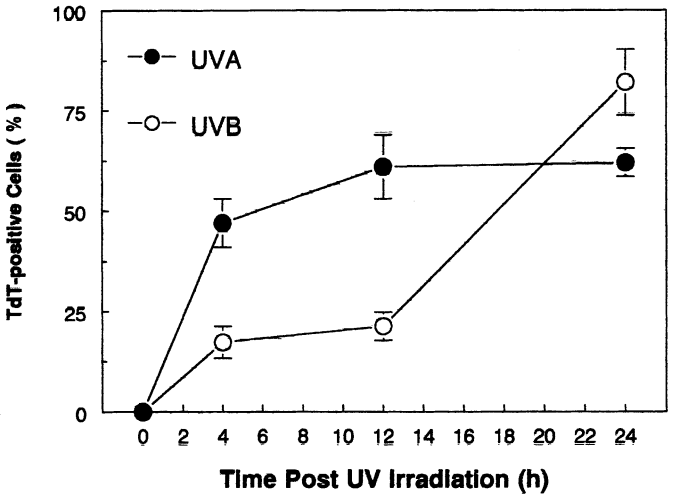

Figure 3. TUNEL assay of UV-irradiated cells. Cells were irradiated with 150 kJ UVA per m^2 or 2 kJ UVB per m^2. Cells were harvested 4, 12, and 24 h post-UV irradiation and tdt-positive cells were quantitated using a TUNEL assay as described in *Materials and Methods*. The results presented are the average of three experiments with each point performed in triplicate.

as compared with the sham control, indicating the occurrence of cell death.

TdT staining and DNA fragmentation in cells exposed to UVA and UVB When cells are exposed to detrimental factors, e.g., DNA damaging agents, apoptosis can be initiated by activation of endogenous nuclear cleavaging enzymes and displays a characteristic DNA ladder, an early sign of apoptosis (Wyllie *et al*, 1984; Pandey *et al*, 1994). TUNEL assay provides a more sensitive approach to detect the early signs of apoptosis compared with the conventional DNA ladder assay (Benassi *et al*, 1997). **Figure 3** shows the TUNEL assays of UVA- and UVB-irradiated cells. A high portion of TdT-positive cells (≈50%) were observed at 4 h post-UVA irradiation, slightly increased at 12 h,

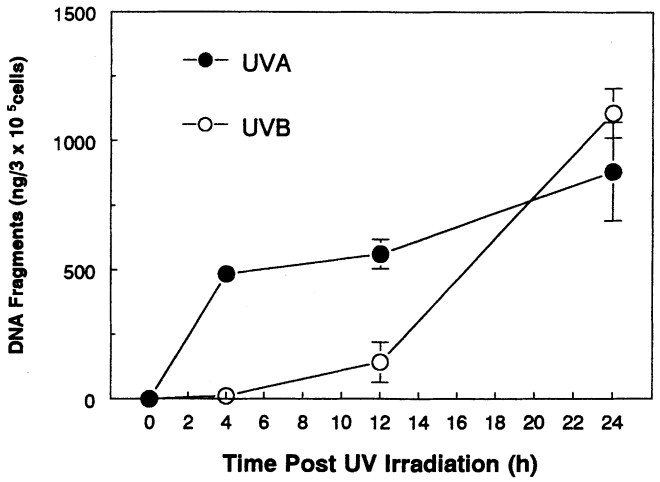

Figure 4. DNA fragmentation assay of UV-irradiated cells. Cells were irradiated with 150 kJ UVA per m^2 or 2 kJ UVB per m^2. Cells were harvested 4, 12, and 24 h post-UV irradiation and fragmented DNA in supernatant was quantitated using a spectrophotofluorometer. The results presented are representative of two experiments with each point performed in triplicate. Both experiments showed a similar trend for UV-induced DNA fragmentation.

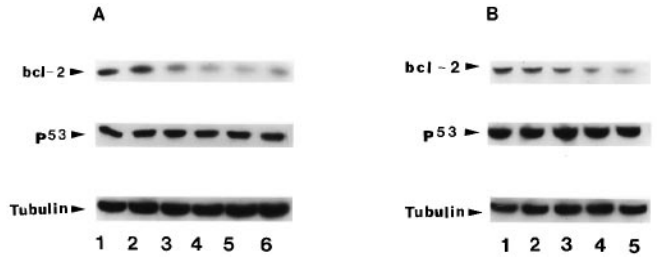

Figure 5. Effects of UVA irradiation on *p53* and *bcl-2* expression. Cells were irradiated with appropriate doses of UVA and harvested at different times post-UV radiation. *P53* and *Bcl-2* proteins were assayed as described in *Materials and Methods*. (*A*) Time course of *p53* and *bcl-2* expression: *lanes 1–6* represent 0, 1, 4, 12, 24, and 48 h, respectively, post-exposure to 150 kJ UVA per m^2. (*B*) Dose–response of *p53* and *bcl-2* expression: *lanes 1–5* represent *p53* and *bcl-2* expression in cells at 24 h post-UVA radiation at doses of 0, 25, 50, 100, and 200 kJ per m^2, respectively. Data presented are representative of three experiments. All three experiments showed consistency for expression of *bcl-2* and *p53* upon UVA irradiation.

and remained constant at 24 h. With cells irradiated with UVB, TdT-positive cells were moderately elevated at 4 h, remained unchanged at 12 h, and sharply increased to ≈80% at 24 h. To corroborate the time course of UV-induced apoptosis, a fluorometric approach was used to quantitate the fragmented DNA in MCF-7 cells. **Figure 4** shows that DNA fragments increased in cells at 4 h post-UVA exposure and then gradually increased at 24 h. In contrast, fragmented DNA did not increase until 12 h post-UVB exposure and sharply increased at 24 h. This experiment was consistent with TUNEL assay, suggesting that UVA induced immediate apoptosis that occurred at 4 h, whereas UVB caused delayed apoptosis that was evident at 24 h. The TUNEL assay was more sensitive than DNA fragmentation assay because ≈20% of TdT-positive cells were observed at 4 h with TUNEL assay, whereas there was no elevation of fragmented DNA within the same time frame.

Effect of UVA irradiation on *Bcl-2* and *P53* expression **Fig 5**(***A***) shows the time course of *bcl-2* and *p53* expression upon UVA irradiation. Tubulin is used as a house keeper gene protein to normalize the *bal-2* and *p53* gene products. Expression of *bcl-2* was downregulated in a time-dependent manner. *Bcl-2* expression was reduced by ≈50% at 5 h post-150 kJ UVA irradiation per m^2 with a maximum downregulation (≈72%) at 24 h. The downregulation of *bcl-2* expression was consistent with the occurrence of immediate apoptosis by UVA as evidenced by TUNEL assay and DNA fragmentation assay (see **Figs 3** and **4**). **Figure 5**(***B***) shows the dose–response of *bcl*-2 and *p53* expression upon

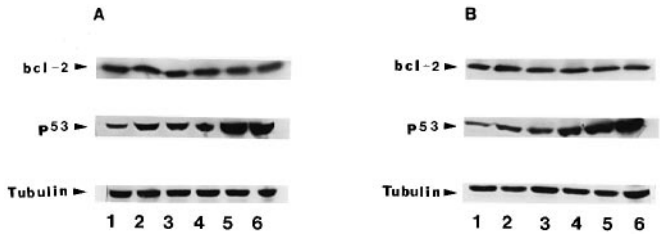

Figure 6. Effects of UVB irradiation on *p53* and *bcl-2* expression. Cells were irradiated with appropriate doses of UVB and harvested at different times post-UV irradiation. *P53* and *Bcl-2* proteins were assayed as described in *Materials and Methods*. (*A*) Time course of *p53* and *bcl-2* expression: *lanes 1–6* represent 0, 1, 4, 12, 24, and 48 h post-exposure to 2 kJ UVB per m^2. (*B*) Dose–response of *p53* and *bcl-2* expression: *lanes 1–5* represent *P53* and *Bcl-2* proteins in cells at 24 h post-UVB radiation at doses of 0, 0.25, 0.5, 1, 2, and 4 kJ per m^2. Data presented are representative of two experiments and both experiments showed similar tendency for expression of *bcl-2* and *p53* upon UVB irradiation.

UVA exposure. Downregulation of *bcl*-2 expression by UVA was dose dependent. A significant reduction of *bcl*-2 expression was observed at 50 kJ UVA per m^2 and high doses of UVA (100 and 200 kJ per m^2) caused a more substantial decrease in *Bcl*-2 levels in MCF-7 cells; however, the levels of *P53* proteins were not affected by UVA.

Effect of UVB irradiation on *P53* and *Bcl-2* expression The results from this experiment showed that UVB irradiation exhibited entirely different actions from UVA in that UVB substantially increased the *p53* expression, but had little effect on *bcl*-2 expression. **Figure 6**(***A***) shows the time course of *bcl*-2 and *p53* expression upon UVB irradiation. Induction of *P53* protein by UVB was time dependent and the maximum expression occurred at 24 h and 48 h with a 6.5- and 4.2-fold increase in *P53* proteins, respectively. The time course of *p53* induction was consistent with the delayed apoptosis induced by UVB (see **Figs 3** and **4**). **Figure 6**(***B***) shows the dose–response of *bcl*-2 and *p53* expression. UVB irradiation induced *p53* expression in a dose-dependent fashion and the *P53* protein levels were increased by more than 6-fold at 2 and 4 kJ UVB per m^2. In contrast to UVA, UVB irradiation did not alter *bcl*-2 expression in MCF-7 cells.

DISCUSSION

UV-induced apoptosis has been reported as sunburn cell formation *in vivo* (Young, 1987). An *in vitro* study showed that apoptosis in response to UVB irradiation was characterized by morphologic change as well as internucleosomal DNA cleavage (Martin and Cotter, 1991). Godar *et al* (1994) reported that all waveband regions of UV light resulted primarily in apoptosis rather than necrosis and that two distinctly different apoptotic cell death mechanisms exist that are UV wavelength dependent. Furthermore, they observed that UVA radiation caused the disruption of membrane permeability and immediate apoptosis, which occurred at less than 4 h post-UVA exposure. UVB and UVC radiation mainly caused delayed apoptosis (usually longer than 20 h). In the latter type of apoptosis, DNA damage in the form of pyrimidine dimers, and probably oxidatively modified DNA bases, could be the major mechanism (Godar and Lucas, 1995). In our study, we observed that both UVA and UVB induced MCF-7 cells to undergo apoptosis over a different time course, as evidenced by morphologic changes and DNA fragmentation. TUNEL assay and quantitation of fragmented DNA showed that apoptosis occurred much earlier in cells exposed to UVA than in cells exposed to UVB irradiation. Our observation on UVA and UVB-induced apoptosis is generally consistent with the results of Codar *et al* (1994). The slight difference in the temporal course may be due to the use of different cell lines, UV lamps (filtered or unfiltered), and analytic approaches.

Bcl-2 protein is encoded by a gene 230 kb in size that gives rise to a 24–26 KD protein that has been localized to intracellular sites of reactive oxygen species generation, including the mitochondria, endoplasmic reticula, and nuclear membranes (Hockenberry *et al*, 1993). The major function of *Bcl-2* appears to inhibit apoptosis caused by a variety of pathologic stimuli (Hockenberry *et al*, 1993; Reed, 1994). The mechanism of *Bcl-2* proteins has not been clearly defined yet; however, it may exhibit two main biochemical properties: (i) it acts in an antioxidant to eliminate reactive oxygen species that may cause damage to DNA and lipids; and (ii) it modulates intracellular Ca^{++} fluxes (Hockenberry *et al*, 1991; Kane *et al*, 1993). Interestingly these two properties match well with the mechanism of UVA irradiation. It is known that UVA radiation generates reactive oxygen species and causes lipids peroxidation (Kane *et al*, 1993), and increases the membrane permeability of white and red blood cells, resulting in the Ca^{++} fluxes (Godar *et al*, 1993, 1994; Godar and Lucas, 1995). In comparison with UVB and UVC, UVA mainly cause oxidative damage to the cell components rather than direct photodamage to DNA. Indeed, recent evidence suggests that oxidative stress may play a common role in the regulation of apoptosis (Buttke and Sandstrom, 1994). Exposure to H_2O_2 was found to be a potent inducer of apoptosis in HL-60 cells (Lennon *et al*, 1991), and the oxygen radical scavengers were able to block nuclear fragmentation and reduce the formation of apoptotic bodies in UV-irradiated HL-60 cells (Verhaegen *et al*, 1995). Over-expression of *bcl-2* functioned to suppress lipid peroxidation and oxidative injuries to other macromolecules (Hockenberry *et al*, 1993). Our data suggest that UVA irradiation-induced oxidative stress may counteract the function of Bcl-2 oncoprotein; however, the exact mechanism by which UVA downregulates expression of *bcl-2* remains unclear. We assume that UVA may either repress the gene expression or accelerate Bcl-2 protein turnover by eliminating oxidatively modified Bcl-2 products.

P53 is a nuclear phosphoprotein and is recognized as a tumor suppressor gene that plays a central role in controlling oncogenic development in humans (Lee *et al*, 1994). It has been reported that the majority of squamous cell carcinomas of the skin contain mutations in *p53* gene, and that these are of the type caused by UV radiation because the specific CC→TT double base change is only known to be induced by UV radiation (Brash *et al*, 1991). It has been reported that, in human skin, exposure to equal erythemogenic doses of UVA, UVB, and UVC increase *p53* expression in a wavelength-specific pattern (Campbell *et al*, 1993). UVC produced *p53* immunostaining confined to the upper epidermis. With UVB, staining was seen throughout the epidermis, whereas with UVA staining predominated in the basal layer. This suggests that the mechanism of response with UVA is different from that seen with UVB and UVC. P53 may bind to or directly activate other cellular proteins required for initiating the apoptosis (Kastan *et al*, 1991). Smith *et al* (1995) reported that P53 may be directly involved in DNA repair by binding to single strand DNA, and that P53-related gene products functioned in the nucleotide excision repair pathway. Cells from P53 deficient mice were unable to undergo apoptosis induced by ionizing radiation (Lowe *et al*, 1993a). These results supported the hypothesis that P53 is directly involved in the DNA repair process. In this study, the *p53* expression pattern was wavelength dependent, e.g., the accumulation of P53 proteins only responded to UVB and not to UVA. This finding strongly suggests that P53 products are exclusively involved in UVB-induced apoptosis. It is known that damaged DNA upregulates *p53* expression, which serves as either a regulator for DNA repair or an inducer for apoptosis. The response pattern of *p53* to UVB was in agreement with our recent findings that UVB induced both pyrimidine dimers and oxidative DNA damage more efficiently than UVA (Zhang *et al*, 1997).

Because UVA mainly increases oxidative stress to cells rather than causes direct DNA damage, the mechanism of the apoptotic action of UVA may be predominantly through the modulation of *bcl-2* expression. More recently, Pourzand *et al* (1997) reported that the overexpression of *bcl-2* inhibited immediate apoptosis by UVA in rat fibroblasts, but not by UVB. They also found that overexpression of *bcl-2* was able to decrease the level of heme oxygenase 1 (HO-1), which is a general response to oxidative stress. Our studies were not only consistent with their observations but also provided more direct evidence that upregulation of *p53* is associated with UVB-induced apoptosis and downregulation of *bcl-2* is associated with UVA-induced apoptosis.

In conclusion, we have demonstrated that UV-induced apoptosis is spectrum dependent and that UVA or UVB initiates the apoptosis by

triggering the different signal transduction pathways. UVA induces immediate apoptosis mainly through downregulation of *bcl-2* expression. In contrast, UVB causes delayed apoptosis, characterized by induction of DNA damage and subsequent accumulation of P53 proteins. The differential regulation of apoptosis and related gene products by UVA and UVB may provide the mechanistic insight into photoaging and photocarcinogenesis.

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