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The Influence of UVA and Visible Radiation on Acute Damage by Short-Wave UVR ($\lambda < 320$ nm)

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The influence of UVA and visible radiation on the acute damage by short-wave ultraviolet radiation (UVR) ($\lambda < 320$ nm) was investigated in human volunteers, using delayed erythema and sunburn cell production as markers of injury. It was found that subsequent exposure to UVA + visible radiation produced a significant reduction of the threshold erythema dose by short-wave UVR, in a dose-dependent manner. Subsequent exposures to varying doses of UVA + visible radiation, as well as to visible light alone failed to influence sunburn cell production. It is concluded that there is a positive interaction between short-wave UVR and UVA in the induction of delayed erythema, but this may not apply to epidermal cell injury. Photorecovery was not observed.

The interaction among UV wavelengths of different energies in causing skin damage has been a subject of growing concern. The acute effects of actinic radiation are due predominantly to wavelengths in the UVB region of the spectrum (290–320 nm). It has been claimed, however, that the cutaneous responses to UVB can be modified by exposure to longer UV wavelengths (UVA, 320–400 nm) or to visible light [1,2]. These observations have significant implications from both a fundamental and clinical standpoint. Although UVA is far less efficient biologically than is UVB, the intensity of UVA in solar radiation reaching the earth's surface is much larger and can be orders of magnitude greater than that of UVB [3]. Theoretically, any such interaction might also be expected to influence the chronic effects of UV radiation (UVR) as well. Furthermore, in various phototherapy protocols, cumulative exposures to several hundred joules/cm^2 of UVA is not unusual, and the impact of such doses on actinic damage in chronically sun-exposed areas has to be seriously considered.

The nature of the UVA and UVB interaction in causing acute skin damage is not fully clear. There seems to be little disagreement that prior exposure to subthreshold doses of UVA renders the skin more sensitive to the erythemogenic effects of UVB [1,2]. This observation has been confirmed by several workers [4,5]. Studies by Ying et al [4] and more recently by Paul and Parrish [6] suggested that this positive interaction could be due to addition of erythemogenically effective energies from both wavebands and not a true synergistic interaction (photoaugmentation) as suggested earlier by Willis et al [2]. Results consistent with photoaddition had previously been published by Sayre et al following combined exposures to the shorter wavelengths 254 nm, 280 nm, and 297 nm from a monochromator [7].

Conflicting reports have appeared, however, concerning the type of interaction when the order of exposures is reversed, viz. UVB followed by UVA. Preliminary studies from this and other laboratories [4,5,8] did indeed show that reversing the sequence of irradiation did not influence the results; the erythemogenic effects of UVB were accentuated. By contrast, Van der Leun and Stoop [1] and later Van Weelden [9] found that the threshold erythema dose (MED) actually increased when exposure to UVB or UVC was immediately followed by UVA. This suggests a negative interaction. They termed this phenomenon photorecovery. More recently, Paul and Parrish [6] also observed a modest elevation in the MED when subthreshold exposures to UVB were followed by UVA, but the increase was not significant.

It is important to determine whether UVA and visible radiation can mitigate or accentuate the damaging effects of shorter UV wavelengths. Experiments were therefore designed to reexamine this question, using histologic criteria as well as erythema.

MATERIALS AND METHODS

Subjects

These were paid healthy Caucasian college students of both sexes between the ages of 18 and 36 years (skin types II and III). Informed consent was obtained. The untanned midback was used as the test site.

Light Sources

A 150-W compact xenon arc solar simulator, equipped with a UVR-reflecting dichroic mirror, was used to provide a broad UVB waveband (290–320 nm). The reflected beam was passed through (a) a 2-mm Schott WG295 filter (50% cutoff at around 290 nm); (b) a UVB band-pass filter with peak transmittance at 300 nm and half-power bandwidth of 25 nm (Infrared Industries, Inc. Waltham, Massachusetts), and (c) a 1-mm Schott UG11 filter to eliminate remaining visible radiation (waveband B, Fig 1). Full-spectrum UVR was obtained from a 200-W high-pressure mercury lamp (Osram HB0 200, Germany) equipped with a UV-transmitting flexible light guide containing a liquid core. The specifications and emission spectrum of this lamp have been published [10].

A 400-W high-pressure mercury lamp, vertically mounted in front of an elliptically shaped reflector was the source of a broadband UVA and visible light. The radiation, after passing through a 7 mm-thick plate of window glass, produced a uniformly illuminated area (± 14%) measuring about 15 × 15 cm at 37.5 cm. The spectral power distribution was determined by an International Light spectroradiometer system (model 782) which showed the spectral lines of mercury with a weaker background continuum extending from 320 nm into the visible region (650 nm). Total irradiance at skin level was 23.9 mW/cm^2; UVA irradiance was 4.5 mW/cm^2. The energy below 320 nm was 0.6 μW/cm^2. There was no measurable transmission below 310 nm.

For studies of sunburn cell formation, 3 broad wavebands with different peaks were isolated from a compact 150-W xenon arc source. The collimated beam, reflected from a UVR-reflecting dichroic mirror, was passed through either a 1-mm Schott WG360 filter (waveband A) or a 3-mm Schott GG385 filter (waveband C). To obtain a more intense and broader visible waveband without UVA, a visible light-reflecting, UVR- and heat-transmitting dichroic mirror was employed, and the reflected beam passed through (a) a 3-mm Schott GG385 and (b) a 2-mm Schott KG4 (infrared absorbing) filter (waveband D). The relative spectral distribution within each waveband is shown in Fig 1. Intensity at skin level was measured by a calibrated thermopile attached to a

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Abbreviations:
HPF: high-power field(s)
MED: minimum erythema dose
MED_B: MED with UVB
MED_{UVR}: MED with UVR
SBC: sunburn cells
UVR: ultraviolet radiation

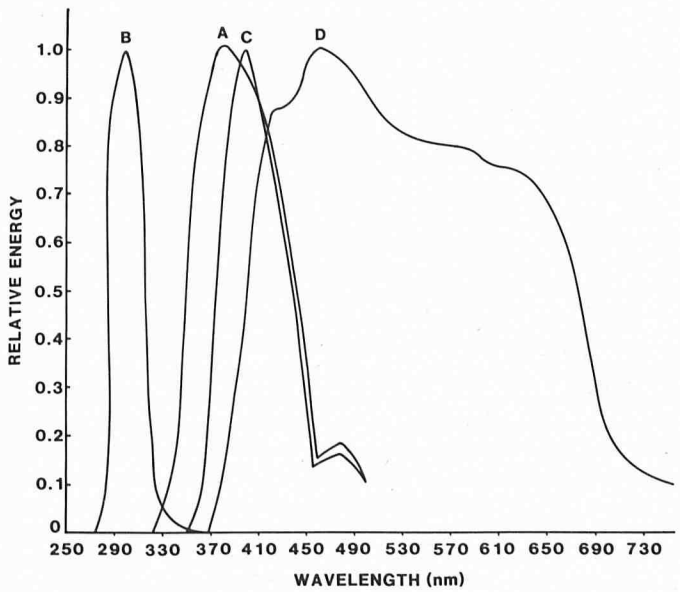

FIG 1. Relative spectral distribution of the isolated wavebands B, A, C, and D.

Keithly millimicrovoltmeter. Intensity measurements of defined wavebands (UVB and UVA) were done with calibrated, cosine-corrected photodiode type detectors equipped with special bandpass filters (models SEE-240 and SEE-010, respectively) and attached to a research radiometer (International Light, Inc., Newburyport, Massachusetts).

Experiments

Influence of UVA and visible light on erythema: The minimum erythema dose (MED) was individually determined by administering a series of exposures to UVB and to UVR from the 200-W mercury source in 25% dose increments to symmetrical areas over the lower back. All exposures were administered in a partially darkened room illuminated by an F40CW fluorescent tube (cool white). Immediately following the exposures, one side of the back was covered by several layers of opaque cloth and the opposite side exposed to UVA and visible radiation from the 400-W filtered mercury source. The subjects were divided into 3 groups; group I received 14.3 J/cm^2, group II received 28.6 J/cm^2, and group III 57.2 J/cm^2 of UVA and visible radiation. The MED with UVB (MED_B) and with UVR (MED_{UVR}) on each side was the smallest dose required to produce visible erythema 24 h later. A *t*-test for paired comparisons was used to test the significance of differences in the MEDs between the opposite sides. Since MEDs show a log normal distribution, the logarithm of the individual values were used in the analysis.

Influence of UVA and visible radiation on sunburn cell production: The 200-W high-pressure mercury lamp was used as the UVR source for induction of sunburn cells (SBC). Dose-response studies were performed initially to determine the dose of UVR required to elicit an appropriate number of SBCs. Subjects were exposed to 0.8 J/cm^2, 1.6 J/cm^2, and 3.2 J/cm^2 of UVR to adjacent sites on the lower back. Superficial shave biopsies (3 × 3 mm) were obtained with a Gillette Blue Blade 22–24 h later from each irradiated site following local xylocaine anesthesia. The specimens were fixed in 10% buffered formalin, processed routinely, sectioned at 6 μm, and stained with hematoxylin-eosin. SBCs were counted at a magnification of 400× in 80–110 random high-power fields (HPF) and the mean number per HPF determined for each specimen. Only 1 of every 10 consecutive sections was counted.

Three adjacent test sites, each measuring 1 cm in diameter, were outlined on the lower back of each subject. Two of the sites were exposed to the same UVR dose (0.8 J/cm^2 or 1.6 J/cm^2). Immediately afterwards, one of the UV-irradiated sites and an adjacent unirradiated normal skin site were exposed to 1 of the 3 wavebands (A, C, or D) shown in Fig 1. The third UV-irradiated site not receiving visible light was covered with several layers of opaque cloth. Two dose levels of visible energy (120 J/cm^2 or 200 J/cm^2) were given with each waveband to different groups of subjects. Shave biopsies were obtained 24 h later from each site and the number of SBCs determined as outlined above.

RESULTS

Influence of UVA + Visible Radiation on the MED

Exposure to UVA and visible radiation did not affect the mean MED_B in the 2 groups of subjects who received the lower dose levels of UVA + visible radiation (groups I and II, Table I). At the highest dose level, however, UVA + visible radiation produced a significant lowering of the threshold UVB dose (group III, Table I). Similar results were obtained for the threshold dose with UVR, except that in this case UVA + visible radiation produced a significant lowering of the MED_{UVR} in both groups II and III (Table I). Furthermore, the magnitude of the reduction in the MED_{UVR} was related to the dose of UVA. This is illustrated in Fig 2 where the difference in the mean log MED_{UVR} ($\overline{D}$) between the opposite sides of each of the 3 groups is plotted as a function of the UVA dose.

Influence of UVA and Visible Radiation on SBC production

SBCs were visualized predominantly in the upper layers of the stratum spinosum. However, 11.5% of the cells were located along the basal and epibasal layers. Their numbers increased linearly with respect to log UVR dose (Fig 3). None of the 3 wavebands (A, C, or D) produced SBCs or had any significant effect on SBC production by UVR, regardless of the doses that were employed. The data for each group were therefore pooled and the results are illustrated in Fig 4.

DISCUSSION

The present findings suggest that there is a positive interaction between wavelengths below 320 nm and UVA + visible radiation when the exposures are given in that sequence, viz. UVR < 320 nm followed by UVA, as evidenced by a significant lowerng of the MED. A change in the MED usually indicates an alteration in susceptibility to acute UVR damage. The observed reduction in the MED for wavelengths < 320 nm was also related to the UVA dose.

In 1949, Blum et al first described an enhancement of recovery from UV irradiation by visible light in animal cells [11]. These authors noted that the normalization of the cleavage rate in the eggs of the sea urchin *Arbacia punctulata* following "short" doses of UVB was greatly accelerated by subsequent exposure to visible light in the 400- to 500-nm region of the spectrum. This observation prompted an investigation to determine whether an analogous phenomenon occurred in human skin. They found that the MED was not altered by subseuqent exposure to an unspecified dose of window glass-filtered sunlight [11]. In contrast, Van der Leun and Stoop showed that a single exposure of 5 h to filtered daylight given immediately after UVB or UVC irradiation resulted in a 25–30% increase in the average MED [1]. Similar findings were subsequently described by Van Weelden who used fluorescent mercury tubes with maximal emission near 365 nm to demonstrate photorecovery of UVC- and UVB-induced erythema by longer wavelengths [9]. In both studies, 40% dose increments were given for the determination of the MED. The dose of UVA was not stated. Since the UVA dose required for this effect was thought to be small, it was suggested that the weak UVA component often present in UVB and UVC sources may in fact be sufficient to produce photorecovery during the initial exposure to the shorter wavelengths [1]. In one of the present experiments where UVA and visible radiation were excluded from the UVB source, however, we were unable to demonstrate this phenomenon, and again observed an accentuation of the erythema response by subsequent exposure to UVA + visible radiation.

It is not known whether the increased sensitivity to wavelengths below 320 nm as measured by a lowering of the MED is also paralleled by enhanced damage to other targets. Since SBCs can also serve as alternative markers of acute UVR injury it was considered worthwhile to determine whether their production can also be influenced by UVA and visible radiation.

TABLE I. *Mean threshold erythema dose with UVR and UVB (mJ/cm² ± SD) in normal skin and in skin subsequently exposed to various doses of UVA + visible radiation*

Group	MED_{UVR}	MED_{UVR} (exposed skin)[a]	Dose of UVA + visible (J/cm²)	*p* (paired *t*-test)	Group	MED with UVB	MED with UVB (exposed skin)	Dose of UVA + visible (J/cm²)	*p* (paired *t*-test)
I (n = 16)	754.6 ± 282.1	764.5 ± 314.2	14.3	N.S.[b]	I (n = 14)	14.5 ± 5.0	14.1 ± 6.0	14.3	N.S.
II (n = 15)	778.5 ± 281.2	723.6 ± 283.7	28.6	<0.01	II (n = 17)	17.0 ± 8.3	17.0 ± 8.0	28.6	N.S.
III (n = 23)	843.4 ± 363.1	732.0 ± 295.9	57.2	<0.005	III (n = 23)	17.4 ± 6.5	14.8 ± 4.4	57.2	<0.001

[a] Side of back exposed to UVA + visible radiation.
[b] N.S. = not significant.

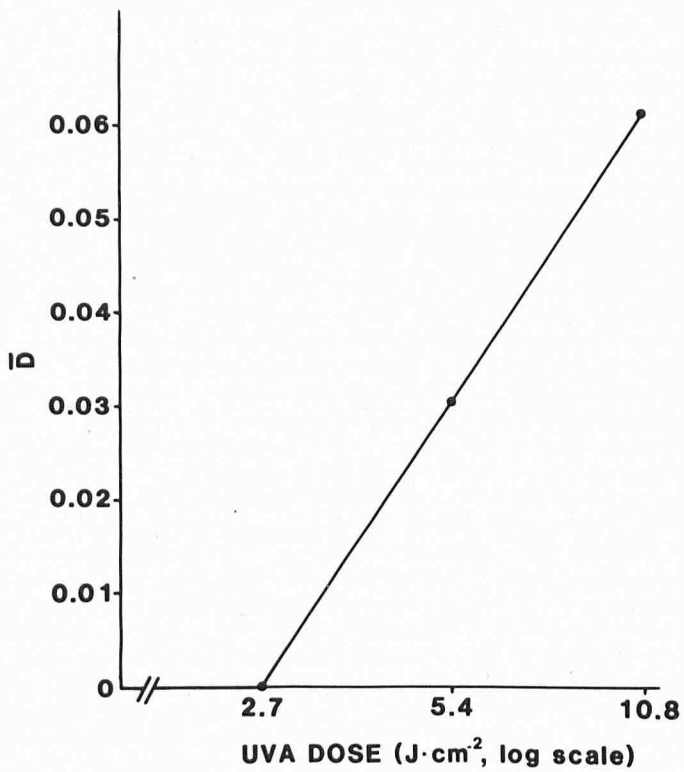

FIG 2. Difference in the mean log MED ($\bar{D}$) for UVR between sites exposed to UVA + visible radiation and unexposed sites, plotted as a function of UVA dose.

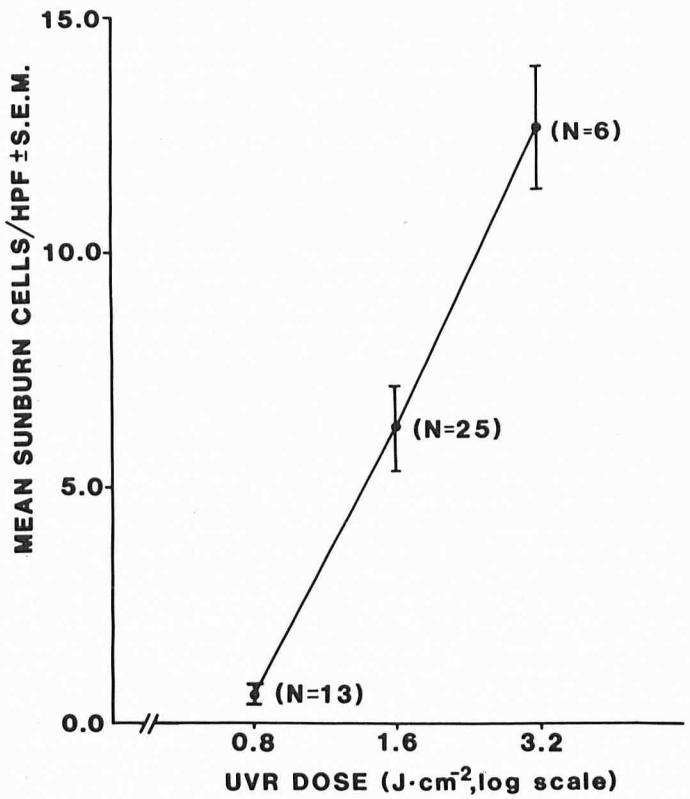

FIG 3. Dose-response for sunburn cell production by UVR.

SBC production was found to be linearly related to the log dose of UVR. A similar dose-response relationship has been described in mouse epidermis [12]. The action spectrum for the production of these cells is limited to wavelengths below 320 nm [12]. The present findings indicate that SBC production is not affected by subsequent exposure to UVA and visible radiation. A slight increase was observed following larger doses of waveband A but this was not significant. Hence the observed interaction may be limited to events that give rise to erythema and may not involve other UV-induced responses. Gange and Mendelson, for example, were unable to show that prior exposure to UVA had any effect on cutaneous responses to UVB using alteration of DNA synthesis and stimulation of ornithine decarboxylase as markers of UVB damage in the hairless mouse [13].

The basis for the observed positive interaction between UVA and UVB is unknown and is likely to remain so until more insight is gained into the mechanism of UVR-induced erythema. The erythemogenic and biologic effects of these spectral

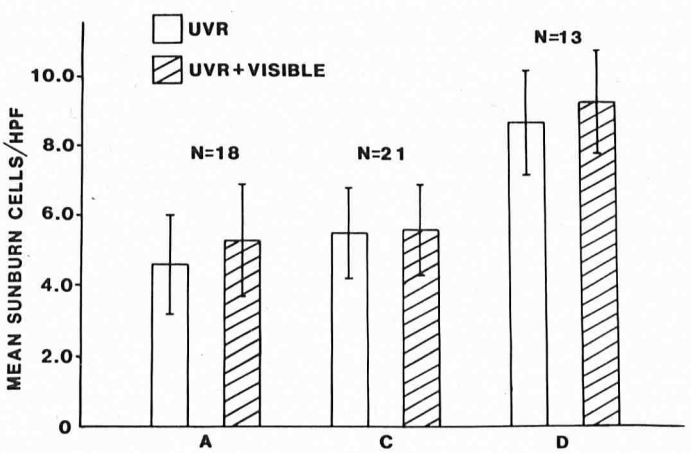

FIG 4. Influence of subsequent exposure to UVA + visible radiation (waveband A) and to visible light alone (wavebands C and D) on sunburn cell production by UVR in 3 groups of subjects.

wavebands differ markedly in several important respects [14,15]. It is also likely that different chromophores are involved in the induction of delayed erythema by UVB and UVA, as suggested in recent action spectra studies by Parrish et al [16]. Nonetheless, several possibilities could be mentioned that may explain the interaction. The theory of simple addition of erythemogenic energies from both wavebands gains support from studies employing subthreshold combinations of broadband UVB and UVA [6]. Unfortunately, we were unable to determine the individual MED with our UVA source because of its low irradiance and hence it is not possible to test the addition theory from the present data. It is worth noting, however, that the mean reduction in the MED for wavelengths < 320 nm was linearly related to the log dose of UVA, which does not in itself suggest simple addition. Clearly, more studies are required to firmly establish photoaddition as the basis for this interaction. Another mechanism by which UVA can adversely influence UVB damage is by interference or inhibition of repair enzymes. Evidence for this comes primarily from studies performed in vitro. Prior exposure of bacteria and of normal human fibroblasts in culture to 365-nm radiation reduces the capacity of these cells to perform unscheduled DNA repair following challenge with UVC [17,18]. There is good evidence to suggest that the cytotoxic effects of UVB and UVC on mammalian and bacterial cells is predominantly due to a DNA lesion, most likely pyrimidine dimer formation [19]. The lethal effects of UVA, especially at high fluences, are believed to be due to additional mechanisms, including inhibition of repair processes and other essential enzymes [20,21]. Whether a similar effect occurs in human skin is currently under study.

REFERENCES

1. Van der Leun JC, Stoop TH: Photorecovery of ultraviolet erythema, The Biologic Effects of Ultraviolet Radiation with Emphasis on the Skin. Edited by F Urbach. Oxford, Pergamon Press, 1969, pp 251–254
2. Willis I, Kligman AM, Epstein J: Effects of long ultraviolet rays on human skin: photoprotective or photoaugmentative? J Invest Dermatol 59:416–420, 1973
3. Bener P: Spectral intensity of natural ultraviolet radiation and its dependence on various parameters, The Biologic Effects of Ultraviolet Radiation with Emphasis on the Skin. Edited by F Urbach. Oxford, Pergamon Press, 1969, pp 351–358
4. Ying CY, Parrish JA, Pathak MA: Additive erythemogenic effects of middle (280–320 nm) and long (320–400 nm) wave ultraviolet light. J Invest Dermatol 63:273–278, 1974
5. Spiegel H, Plewig G, Hofmann C, Braun-Falco O: Photoaugmentation. Arch Dermatol Res 261:189–200, 1978
6. Paul BS, Parrish JA: The interaction of UVA and UVB in the production of threshold erythema. J Invest Dermatol 78:371–374, 1982
7. Sayre RM, Olson RL, Everett MA: Quantitative studies on erythema. J Invest Dermatol 46:240–244, 1966
8. Kaidbey KH, Kligman AM: Further studies of photoaugmentation in humans: phototoxic reactions. J Invest Dermatol 65:472–475, 1975
9. Van Weelden H: Photorecovery in human skin, Lasers in Photomedicine and Photobiology. Edited by R Pratesi, CA Sacchi. Berlin/Heidelberg/New York, Springer-Verlag, 1980, pp 129–133
10. Plewig G, Hofmann C, Braun-Falco O, Nath G, Kreitmair A: A new apparatus for the delivery of high intensity UVA and UVA + UVB irradiation, and some dermatological applications. Br J Dermatol 98:15–24, 1978
11. Blum HF, Loos GM, Price JP, Robinson JC: Enhancement by "visible" light of recovery from ultraviolet irradiation in animal cells. Nature 164:1011, 1949
12. Woodcock A, Magnus IA: The sunburn cell in mouse skin: preliminary quantitative studies on its production. Br J Dermatol 95:459–468, 1976
13. Gange RW, Mendelson IR: Photoaugmentation in the hairless mouse: a study using ornithine decarboxylase activity and alteration of DNA synthesis as markers of epidermal response. J Invest Dermatol 77:393–396, 1981
14. Parrish JA, Anderson RR, Urbach F, Pitts D: Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Longwave Ultraviolet. New York, Plenum Press, 1978 pp 107–139
15. Kaidbey KH, Kligman AM: The acute effects of longwave ultraviolet radiation on human skin. J Invest Dermatol 72:253–256, 1979
16. Parrish JA, Jaenicke KF, Anderson RR: Erythema and melanogenesis action spectra of normal human skin. Photochem Photobiol 36:187–191, 1982
17. Tyrrell RM, Webb RB: Reduced dimer excision following near ultraviolet (365 nm) radiation. Mutat Res 19:361–364, 1973
18. Smith PJ, Paterson MC: Lethality and the induction and repair of DNA damage in far, mid or near UV-irradiated human fibroblasts: comparison of effects in normal, xeroderma pigmentosum and Bloom's syndrome cells. Photochem Photobiol 36:333–343, 1982
19. Coohill TP, Jacobson ED: Action spectra in mammalian cells exposed to ultraviolet radiation. Photochem Photobiol 33:941–945, 1981
20. Danpure HJ, Tyrrell RM: Oxygen-dependence of near UV (365 nm) lethality and the interaction of near UV and x-rays in two mammalian cell lines. Photochem Photobiol 23:171–177, 1976
21. Webb RB: Lethal and mutagenic effects of near-ultraviolet radiation, Photochemical and Photobiological Reviews, vol 2. Edited by KC Smith. New York, Plenum Press, 1977, pp 169–261