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Action Spectra of Apoptosis Induction and Reproductive Cell Death in L5178Y cells in UV-B Region

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It is important to determine the action spectrum of UV-B radiation contained in the sunlight to estimate the risk of skin cancer. We have investigated action spectra for induction of apoptosis and reproductive cell death in L5178Y cells using the Okazaki Large Spectrograph at NIBB. L5178Y cells were exposed to light at different wavelengths in UV-B or UV-A region. Frequencies of apoptosis induction and reproductive cell death were determined by counting cells with chromatin condensation, and by the colony formation assay, respectively. The measured sensitivity spectra for the two end-points were in very good agreement. Sensitivity decreased steeply with increase of wavelength in UV-B region and remains nearly constant in UV-A region. The action spectra were also slightly steeper than that for the minimum erythematic dose (MED), but very similar to the light absorption spectrum of DNA in UV-B region. On the other hand, the spectra for both endpoints were similar to MED spectrum but not DNA spectrum in the UV-A region. Also different time-course and morphological difference of apoptosis were found between UV-B (long time, fragmentation) and UV-A (short time, shrinkage) region. These results suggest that DNA damage induced by UV-B light triggers apoptosis and reproductive cell death, but other damaged targets (membrane, protein and so on) trigger these effects in UV-A region.

Key words: apoptosis, reproductive cell death, action spectrum, L5178Y cells, monochromatic UV-B light

INTRODUCTION

It is well known that stratospheric ozone is being depleted, resulting in the possibility of an increase in the amount of UV-B reaching the ground. It is therefore essential that complete UV-B action spectra for a variety of biological responses of human, animal, and plant systems be obtained. Action spectroscopy has a long history and is of central importance in photobiological studies. Combining these action spectra with the incident UV-B spectra on the ground can give rise to solar UV effectiveness spectra [1, 2, 3] that, in turn, can give rise to estimates of various effects. But little action spectroscopy has been reported recently; even its importance been has suggested [4].

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It is important to determine the action spectrum of apoptosis induction by UV-B radiation contained in natural sunlight to estimate the risk of skin cancer. We have investigated an action spectrum for the induction of apoptosis in L5178Y cells.

MATERIALS AND METHODS

The mouse lymphoma L5178Y cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics. Cells were collected, resuspended in PBS^-, transferred into a small plastic cup covered with a UV short-cut filter to reduce contaminating shorter wavelengths light just before light exposure, and exposed to the light under room temperature.

For colony formation assay, exposed cells were diluted and mixed with the medium containing 0.25%

agarose, and seeded in Petri dishes to provide approximately 100 survivors per dish which covered with the medium solidified by 0.5% agarose. The cells were cultured for 10 days in an incubator. The number of survivors were counted as a number of cell colony under a stereomicroscope without any fixation and staining.

To detect apoptosis, exposed cells were resuspended with the medium, and incubated for 5 or 20 hours. The cells were collected, fixed with 1% glutaraldehyde, and stained with Hoechist 33342 dye. After that, apoptotic cells were counted under a fluorescent microscope.

Ultraviolet light generated from a 30 kW Xenon short-ark lamp and selected by double-blazed monochrometers at the OLS[5] in the NIBB, Okazaki, was used. A light-condensing lens was used to obtain higher intensity light when cells were exposed to longer wavelength lights; the light intensities were varied according to the wavelengths used. A reflecting mirror placed at just above the sample was used to obtain a vertical light.

RESULTS

Apoptosis induced by UV

The incidence of chromatin condensation, formation of a DNA ladder and production of apoptotic bodies were observed following a 5 hr of post-incubation period after the UV-A light (or > 313 nm) exposure. Chromatin condensation was detected by an observation under a fluorescence microscope. Some cells showed a granular-type chromatin condensation, however, a large numbers of cells showed a homologous chromatin condensation. DNA-ladder formation was detected by agarose gel electrophoresis. Clear DNA-ladders as well as positive control cells were obtained. Apoptotic sub-G1 fractions were also detected by a flow cytometer.

Similar results were found when cells exposed to UV-C (or < 290 nm) light. However, the apoptotic change could be found after 20 hr of post-incubation, and the major product in the morphological type was the induction of granular-type chromatin condensation.

Action Spectra

Sensitivity for the induction of apoptosis (AI) was obtained as an inversed value of the exposure that gives induction of apoptosis in 10% cells in total for each wavelengths light. The values were normalized to that at 300 nm (Fig.), and an action spectrum for apoptosis was obtained. The sensitivity decreased along with an increase in the wavelength in the UV-B region (280-320 nm), but no more decrement was observed in the UV-A region (>320 nm). A 1000-times higher exposure was required to obtain the same AI value by UV-A light compared that at 300 nm.

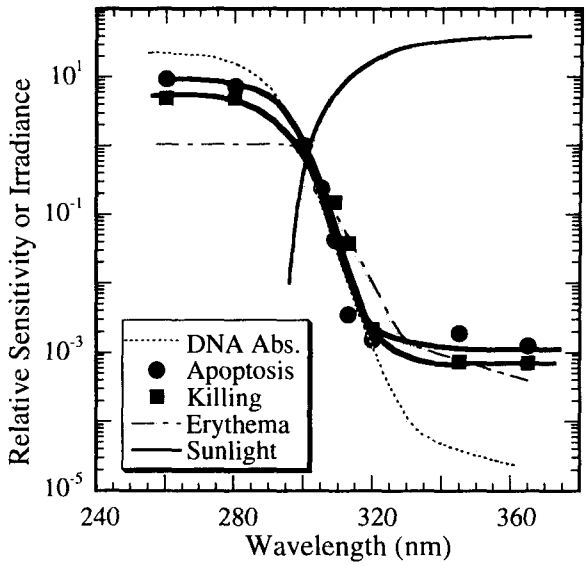

Fig. Spectra of sensitivities in apoptosis or reproductive cell death, with spectra for DNA absorption Minimum Erythema and Solar UV irradiance. Sensitivities were obtained as an inverse value of the exposure at 10% cells in the total cells induce the apoptosis (●), or surviving cells(■). DNA absorption (dotted line)[6], minimum erythema dose (broken line)[7] and solar irradiance (solid line)[8], are taken from literatures.

Sensitivity of reproductive cell death (SF) was also measured as a loss of colony forming ability. The exposures that gives 10% survivors were measured at each wavelengths corresponds to that for apoptosis. The values were normalized again to that at 300 nm, and the inversed value was obtained as the action spectrum of the reproductive cell death, as well as apoptosis. The action spectrum was very similar to that for apoptosis.

DISCUSSION

We could confirm all three major characteristics of apoptosis for both UV-A and -C light. However, a different morphological type of apoptosis between UV-A and -C was observed; *i.e.*, homogeneous chromatin condensation and granular chromatin condensation were found to be major products, respectively. A difference in the time-course of induction of apoptosis was also found. Cells exposed to the UV-A lights showed apoptotic change within several (~5) hours. In contrast, a longer time (~20 hr) was required when cells were exposed to the UV-C lights. We can divide the UV-induced

apoptosis into at least two different types: 1) short-wavelengths type and 2) long-wavelengths type. Similar results involving three different types of apoptosis which could be categorized by the time-course have been reported[9]. In the category, the most quick apoptotic change, immediate pre-programmed apoptosis, was found within 20 minutes. We could not count those cells, because we needed more than 0.5 hour for such high exposure. Cells which experienced very high exposure (~1 MJ/m^2) of UV-A light may showed cell lysis during the exposure.

The morphological type of chromatin condensation by UV-A light was mixed; a small number of the cells showed the fragmentation of cell nuclei, and a large number of cells showed shrinkage of total cell body with the homogeneously condensed cell nuclei. This long wavelengths type apoptosis was found after 5 hours of post-incubation. This may have been caused by plasma membrane damage, and may correspond to intermediate-apoptosis[9]. The morphological types of apoptosis by UV-C light and time-course are very similar to that by ionization radiation. This short-wavelength type apoptosis is caused by DNA damage, and may correspond to delayed-apoptosis.

The measured sensitivity spectra for the two endpoints were in very good agreement. Sensitivity decreased steeply with increase of wavelength in below 320 nm and remains nearly constant in UV-A region. The action spectra were also slightly steeper than that for the minimum erythematic dose (MED)[7], but very similar to the light absorption spectrum of DNA in UV-B region[6] (Fig.). This similarity strongly suggests that DNA damage induced by UV-B or -C light would be a trigger of apoptotic changes, because at least a large part of induction of apoptosis by ionization radiation for cells is triggered by DNA damage[10]. On the other hand, the spectra for both endpoints were similar to MED spectrum but not DNA spectrum in the UV-A region. Apoptosis induced by UV-A light or a longer wavelengths region in UV-B light may be induced by a pathway that is directly related to membrane damage. The relative sensitivity at wavelengths greater than 320 nm did not show any specific dependency on the wavelength. Also different in time-course and the morphological difference of apoptosis were found between UV-B (long time, fragmentation) and UV-A (short time, shrinkage) region. These results suggest that DNA damage induced by UV-B light triggers apoptosis and reproductive cell death, but other damaged triggers (membrane, protein and so on) trigger these effects in UV-A region.

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REFERENCES

[1] Furusawa, Y., K. Suzuki, and M. Sasaki (1988) UV-B dosimeters for monitoring the risk of skin cancer by natural sunlight. *Photomed. Photobiol.* 10: 201-204.

[2] Furusawa, Y., L. E. Quintern, H. Holtschmidt, P. Koepke, and M. Saito (1998) Determination of erythema-effective solar radiation in Japan and Germany with a spore monolayer film optimized for the detection of UVB and UVA--results of a field campaign. *Appl Microbiol Biotechnol* 50: 597-603.

[3] Munakata, N., F. Morohoshi, K. Hieda, K. Suzuki, Y. Furusawa, H. Shimura, and T. Ito (1996) Experimental correspondence between spore dosimetry and spectral photometry of solar ultraviolet radiation. *Photochem Photobiol* 63: 74-78.

[4] Coohill, T. P. (1991) Action spectra again? *Photochem Photobiol* 54: 859-870.

[5] Watanabe, M., M. Furuya, Y. Miyoshi, Y. Inoue, I. Iwahashi, and K. Matsumoto (1982) Design and performance of the Okazaki Large Spectrograph for photobiological research. *Photochem Photobiol* 36: 491-498.

[6] Setlow, R. B. (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proc Natl Acad Sci USA* 71: 3363-3366.

[7] McKinlay, A.F. and B. L. Diffy (1987) A reference action spectrum for ultraviolet induced erythema in human skin. CIE J 6: 17-22.

[8] Ito, T. (1993) UV-B observation network in the Japan Meteorologicl Agency. *In: Frontiers of Photobiology*, Eds. by Shima A. et al., Excerpta Medica, Amsterdam. pp. 515-518.

[9] Godar, D.E. (1999) UVA1 radiation triggers two different final apoptotic pathways. *J Invest Dermatol* 112: 3-12.

[10] Aoki, M., Y. Furusawa, and T. Yamada (2000) LET dependency of heavy-ion induced apoptosis in V79 cells. *J Radiat Res* 41: 163-175.