A-4.6 Changes in the tissue damages caused by UV-B with various irradiation conditions.

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Abstract To examine dose-effect relationship between longer wave-length UV and cellular damage, we have devised an UV-applicator with shutters controlled electrically, which enabled various irradiation conditions. With this applicator, we observed the effects of low dose UVB exposure. We noticed that detachment of cells from matrix plate, which seemed to be related to apoptosis. This detachment was dependent to dose, but not to exposure pattern. Effects of the exposure to the 30J/m 290nm UV was almost the same within the range from 0.6 μ W to 25 μ W. Production of thiobarbituric acid reactive substances (TBA-RS) was elevated in the medium of cultures which had been exposed to UVB and had caused the detachment.

Key Words UV-B, Oxidative stress, TBA-RS, apoptosis, dose-dependency

1. Introduction

Intencitity of UVs in natural environment changes with time. We ordinarily obtain exposure dose as total energy shed with UVs. But, is the effect of high intensity short time exposure the same as the effect of low intensity long time exposure, when thier total energy is equal? To answer the question, we observed the changes in culture cells exposed to UVB with an UV applicator, with which we can applicate UV with distinct wave length, intensity, and time pattern.

2. Methods

i. Sample preparation KB cell originated from human laryngeal tumor was maintained in MEM supplimented with 10% FCS. Semiconfluent cultures in 35mm dish were exposed to UVB. The dish was washed two times with 2 ml of Hanks' salt solution, added with 0.7ml of Hanks' salt solution, and exposed to UVB. After exposure, medium was changed to MEM containing 10% FCS, and the dish was placed in CO_2 incubator.

ii. UV applicator Our applicator has 2 slots for 35mm culture dish and sheds UV with dintinct wave length with resolving power of 17nm. Intensity of UV can be controlled with ND filter referring to the dose monitor.

iii. Estimation of thiobarbituric acid reactive substance (TBA-RS) was carried out as

described by Morlière et al.[1)]

3. Results

i. We observed the detachment of cells from cell matrixes (Fig. 1), which seemed to be related to apoptosis.[2),3)] The phenomenon was distinct and reproducible. We expressed the extent of the phenomenon with the "detachment scale" (Fig. 1). The "detachment" was caused by short wave length UVB and the proportion of cells that detached was dose dependent with narrow energy range.

ii. We compared the effect of exposure pattern using the "detachment" phenomenon as an indicator. Fig. 2 indicates that the extent of changes caused by 290nm 30J/m was not distinguished between 6mW/m x 5000sec and 250mW/m x 120sec.

iii. Intermittent exposure was not distinguished with continuous exposure during this intensity range. For instance, there was no observable change between 290nm 100mW/m x 300sec continuous exposure and 150 times recurring exposure of 2sec on and 5sec off.

iv. We estimated TBA-RS in the exposed cultures. (Fig. 3) With strong exposure such as 200J/m, TBA-RS content was elevated after one hour after exposure.

v. At twelve hours after exposure, when "detachment" was completed, TBA-RS content in the cell was slightly decreased (Fig. 4) and the amount in the medium was extremely increased (Fig. 5).

4. Discussion

i. With "detachment" phenomenon as an indicator, we observed that total enegy is important during 6-250mW/m and that the difference in intensity had no observable effect.

ii. Mechanism and importance of "detachment" phenomenon should be studied further.

iii. TBA-RS excreted into the medium seems to relate to oxidative stress causing circulating metabolites. Observation in detail of time course and extent of TBA-RS and relating metabolites is needed.

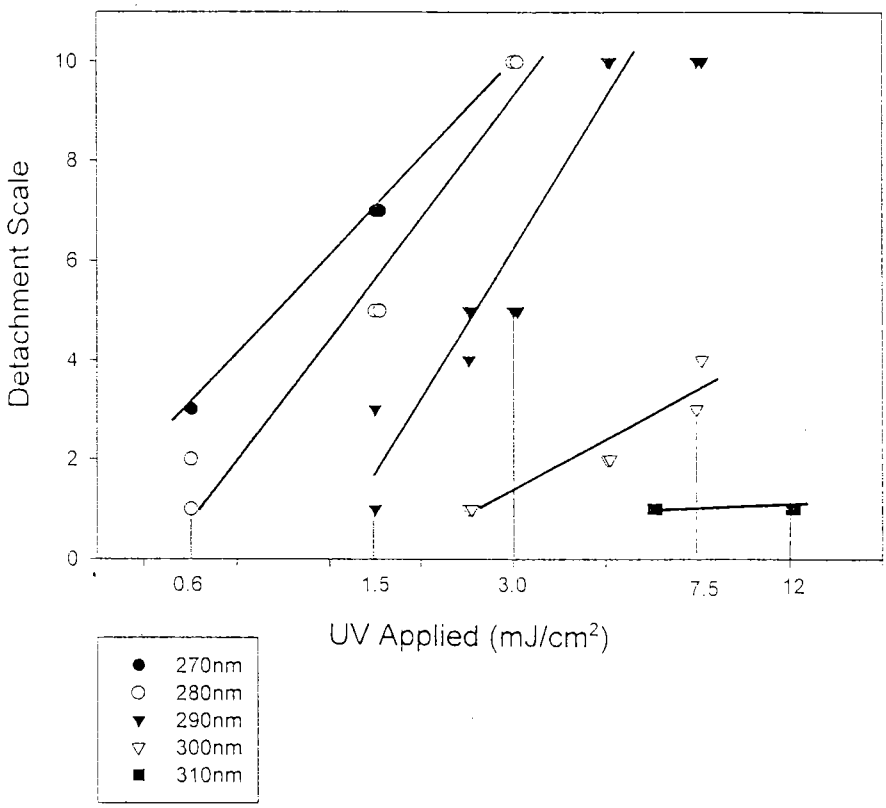
Reference

1) Morlière P, Moysan A, Santus R, Hüppe G, Mazière JC, Dubertret L. (1991) UVA-induced lipid peroxidation in cultured human fibroblasts. Biochim Biophys Acta 1084, 261-268.

2) Zhuang L, Wang B, Shinder GA, Shivji GM, Mak TW, Sauder DN (1999) TNF receptor p55 plays a pivotal role in murine keratinocyte apoptosis induced by ultraviolet B irradiation. J Immunol 162, 1440-1447

3) Barber LA, Spandau DF, Rathman SC, Murphy RC, Johnson CA, Kelly SW, Hurwitz SA, Travers JB (1998) Expression of the platelet-activating factor receptor results in enhanced ultraviolet B radiation-induced apoptosis in a human epidermal cell line. J Biol Chem 273, 18891-18897.

Fig. 1 Dose and Wave Length Dependency of the Effect



Examples of the Detachment Scale

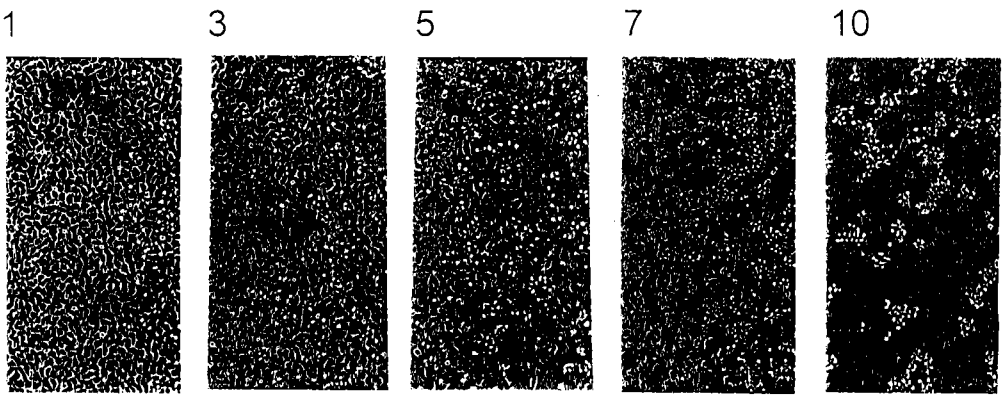
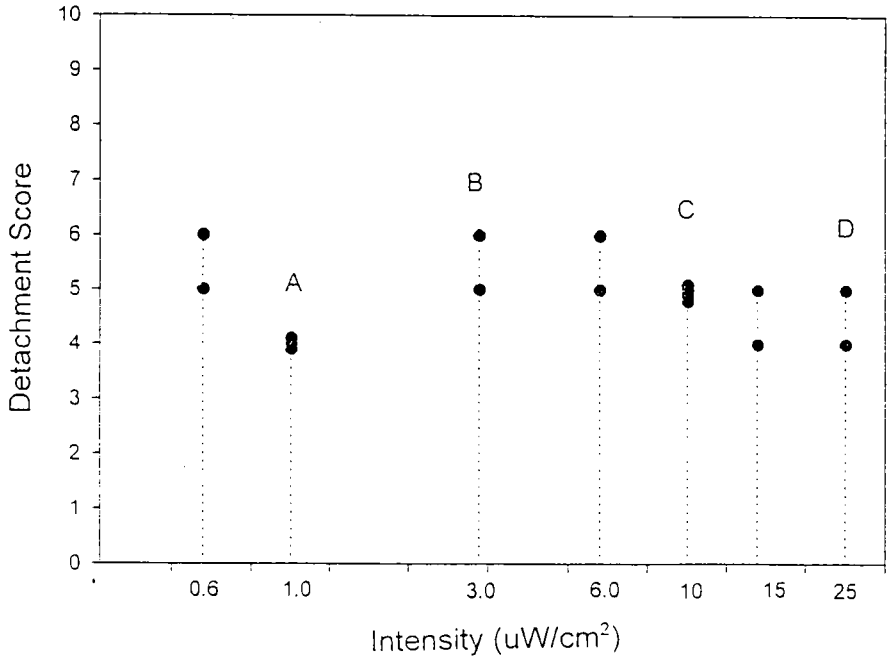


Fig. 2

Cell Detachment Caused by 290nm 30J/m UV with Various Intensities and Durations



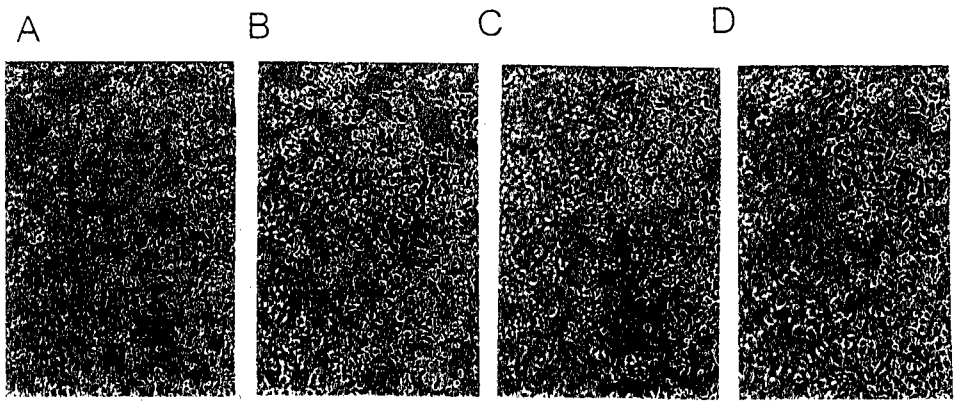


Fig. 3 TBA-RS Production after Exposure to the 200J/m² UV

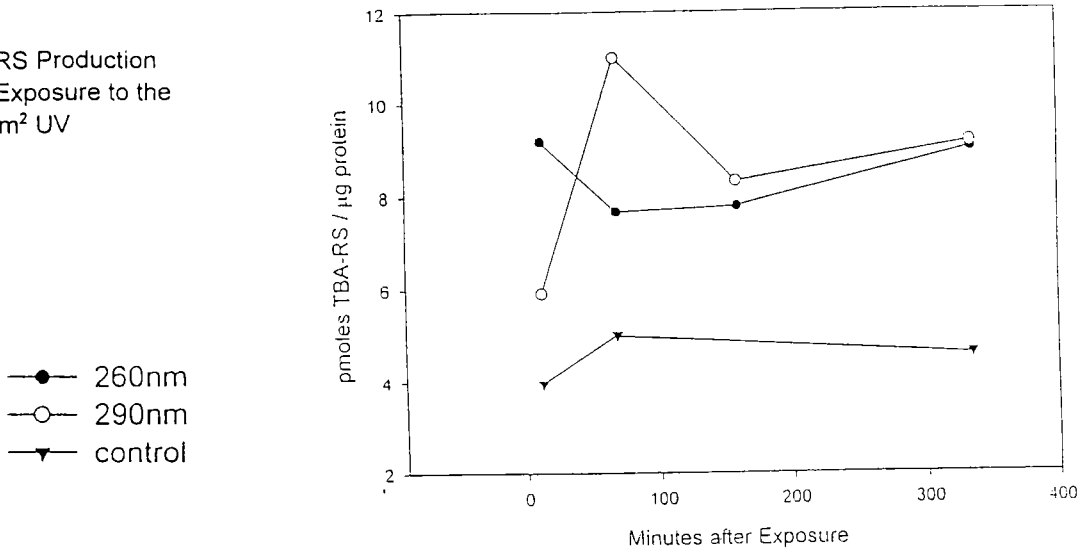
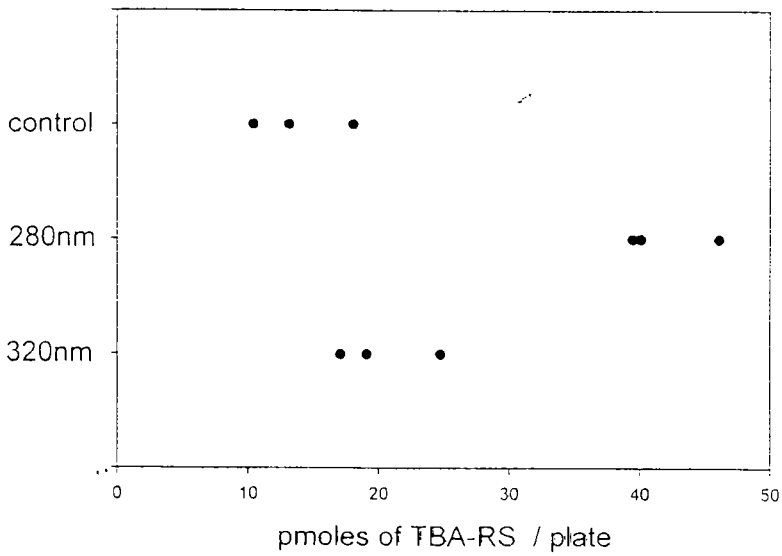


Fig. 4 TBA-RS Excreted into Medium from KB Cells at 12 hours after UV Exposure



Cells were exposed to 10 µ W/cm² UV for 5 minutes.

Fig. 5 TBA-RS Content in KB Cells at 12 hours after UV Exposure