A-4.2.3 Effects on Photoallergy

Contact Person Yamada, Hirotomo
Department of Occupational Diseases,
National Institute of Industrial Health,
Ministry of Labor.
21-1, Nagao 6 chome, Tama-ku, Kawasaki, 214 Japan
Tel +81-044-865-6111 Fax +81-044-865-6116

Total Budget for FY1990-FY1992 29,370,000 Yen

Abstract

This study was designed to estimate risks caused by ultraviolet radiation (UVR) including photoallergy by detecting changes in proteins of human skin cells. Thus far, Gel electrophoresis of proteins from the cells harvested after radiation of shorter wave length UVB has shown increases in several protein bands, which are possible candidates of antigens which cause immune responses, such as photoallergy.

Effects of UVR on the cells exposed to heavy metals (Hg, Cd and Zn) were studied. The double exposures to UV and a metal showed much greater lesions than simple sums of those obtained by exposure to each factor and also induced specific proteins. This means that cellular damages are determined not only by energy (joules) and wavelength (nm) of UVB but seriously affected by co-existence of other enviromental factors.

Key Words Monochromatic Radiation, Human Skin Cells, Heavy Metals, Photoallergy, Electrophoresis

1 Introduction

Ozone plays an important role in protecting living things from lesions and death due to absorption of solar ultraviolet radiation (UVR). But UVR on the surface of the earth and physical lesions from UVR are suspected to have increased in the time period since ozone destruction by a chlorine-mediated mechanism and the springtime ozone hole in polar regions were reported[1]. The decrease in ozone results in the increase in ultraviolet with wavelength between 280 to 315 nm (VVB) on the earth. UVB has been known to have the strongest erythematic effect upon human skin and recognized to cause more serious lesions like photoallergy.

2 Research Objective

This research was designed to study the formation of photoantigen by UVR in human skin cells. Controlling the energy (joules) and wavelength (nm) of UVB, changes in cellular proteins were studied.

3 Research Method

Monochromatic radiation was obtained from a xenon lamp in combination with a monochromator. Cells of NB1RGB (normal human skin fibroblast) or NCTC 2544 (human epitherial cells) were washed twice with phosphate-buffered saline and irradiated with out phosphate-buffered saline with a single dose of UV.

Cells were disrupted by sonication in 20 μl of 0.25 M Tris-HCl, pH 7.5. Insoluble materials were sedimented at 15 krpm for 5 min. in a microfuge. The supernatant

was used for SDS-7.5 % PAGE[2] and protein bands were detected by silver staining[3].

Cellular proteins were labeled with 20 μC of ^{35}S-Methionine for 20 hours. Pre-label means cellular proteins were labeled before UVR. In post-label, they were labeled after irradiation. Electrophoretic analysis of cell lysates was performed by fluorography.

4 Results

Fig 1 and 2 shows dose effects of UVB on protein. For dead cells are floating and removed by washing, recovered protein at 20 hours after UVR reflects amount of living cells. UVR at 280 nm reduced the protein linearly in semi-log graph. UVR at 300 nm less than 400 J/m² did not decrease protein recovery. Light at 320 nm had no effect on protein even at 744 J/m². This means that the shorter UV is more harmful to the cells.

Fig. 1 NCTC2544

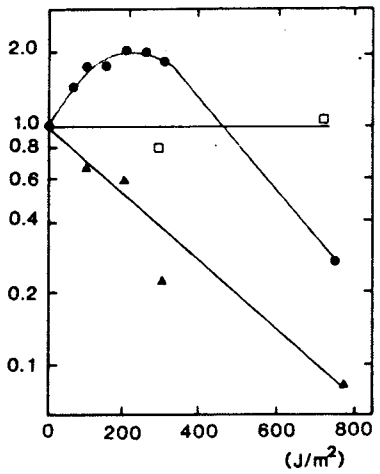


Fig. 2 NB1RGB

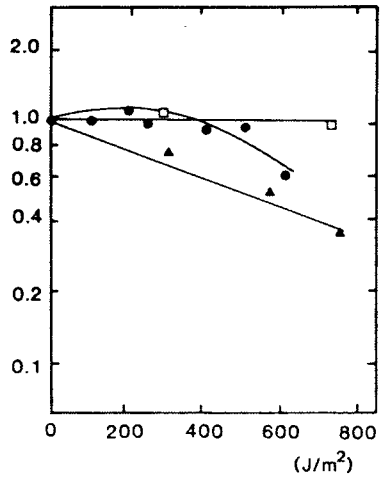


Fig. 3 NB1RGB

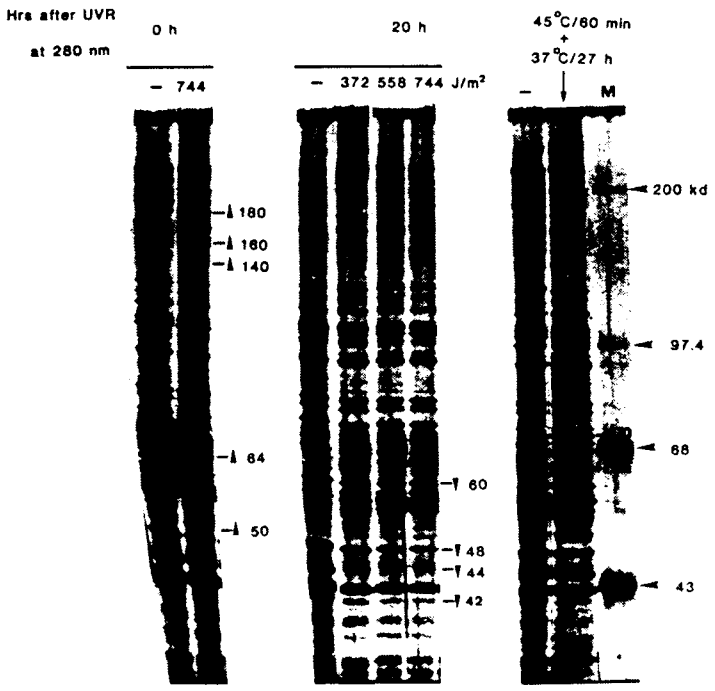
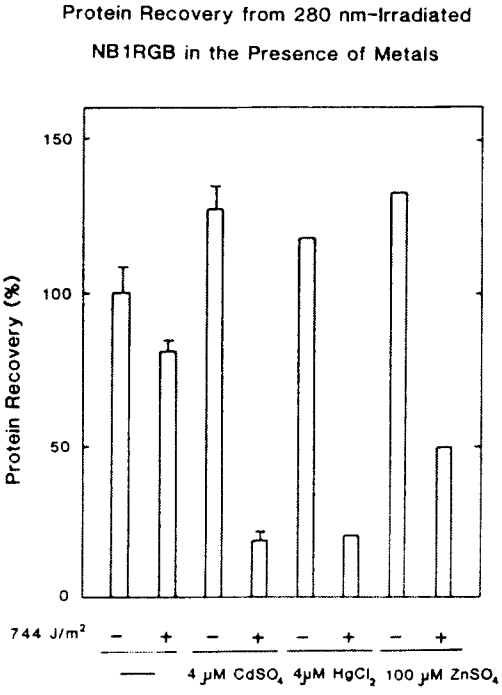


Fig. 4



A gel electrophoresis of protein from the cells harvested immediately after 744 J/m² UVR at 280 nm shows increases in 180, 160, 140, 64 and 50 kd bands, which disappeared within 20 hours (Fig 3). The changes in bands do not seem due to newly synthesized proteins. They are considered due to mobility shift of cell components with some modifications by UVR.

Fig. 5

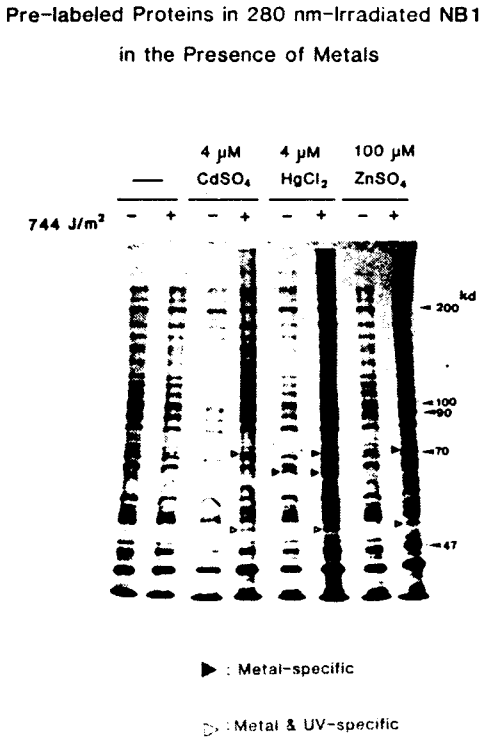


Fig. 6

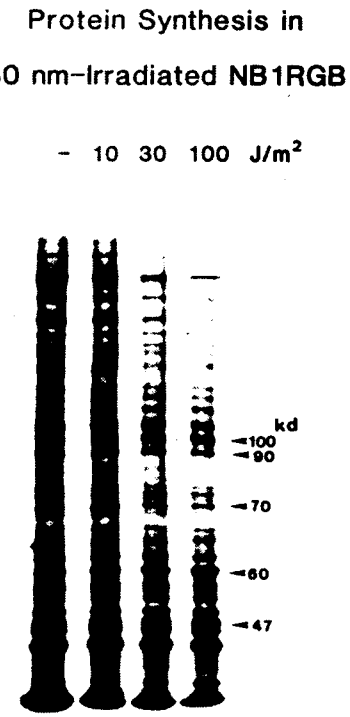


Fig 4 shows effects of UVR on the cells exposed to heavy metals. The cells were irradiated after they were labeled with ^{35}S-Methionine in the presence of 4 μM $CdSO_4$, 4 μM $HgCl_2$ or 100 μM $ZnSO_4$. The labeled cells were immeadiately harvested after UVR. Recovered protein was decreased when the cells exposed to one of the heavy metals were radiated. This suggests that a double exposeure to UV and a heavy metal is more harmful than a single exposure to each of them. Metal-specific (△) and metal and UV-specific (▲) proteins were observed when extracts of the cells were analysed by SDS-7.5 % PAGE (Fig 5). Protein synthesis of NB1RGB was reduced by UVR at 30 J/m² (Fig 6), which is much less than that for changes of pre-labeled protein (Fig 3).

5 Discussion

Gel electrophoresis of proteins from the cells harvested after radiation of shorter wave length UVB has shown increases in several protein bands. The proteins modified by UVR (Fig 3) must be degradated as abnormal proteins in normal living cells. But they may stimulate the immune system when they leak out of the cells or death of the cells sets them free. They seem candidates of antigens which cause immune response, photoallergy.

Effects of UVR on the cells exposed to heavy metals (Hg, Cd and Zn) were studied. The double exposures to UV & a metal showed much greater lesions than simple sums of those obtained by exposure to each factor and also induced specific proteins. Cellular damages seem to be determined not only by energy (joules) and wavelength (nm) of UVB but seriously affected by co-existence of other enviromental factors. This suggests that a physiological condition of of skin cells is critical to reactions of the cells to UVB.

Reference

1) M.R. Schoeberl and D.L. Hartmann (1991) Science 251, 46-52
2) U.K.Leammli (1970) Nature 227, 680-685
3) F.Otsuka, S.Koizumi, M.Kimura and M.Ohsawa (1988) Anal.Biochem. 168, 184-192