A-4.3 Studies on protein changes in UV-irradiated human skin cells

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Abstract

Specific inhibition of metallothionein (MT) induction by monochromatic UVB lights was studied in human skin cells and HeLa cells. When the cells were irradiated with 280-290 nm UV lights even at low doses which did not affect total protein synthesis, the induction of MTs by heavy metals and dexamethazone was immediately inhibited. Northern hybridization showed level of Cd-induced MT-mRNA was suppressed markedly after UV irradiation at 280nm. The inhibition of MT synthesis caused by suppression of MT-mRNA deprived the cells of resistance to heavy metals. The result suggests that at least a part of toxic effects of UVB irradiation could result from the inhibition of MT induction.

Key Words UVB Irradiation, Human Skin Cells, Metallothionein, Suppression of mRNA, Heavy Metals,

1. Introduction

Ozone plays an important role in protecting living things from lesions and death due to absorption of solar ultraviolet. But UV on the surface of the earth and physical lesions from UV 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. The decrease in ozone results in the increase in ultraviolet with wavelength between 280 to 315 nm (UVB) on the earth. UVB has been known to have the strongest erythematic effect upon human skin.

To investigate toxicity of UV on human cells, effects of monochromatic UV (260-320 nm) on protein synthesis have been examined in human skin cells. UV irradiation at 260-290 nm was observed to inhibit total protein synthesis. Synthesis of metallothioneins (MTs) was inhibited most effectively among those of cellular proteins. MTs are small proteins rich in cystein residues, through which they bind heavy metals. They have been suggested to play some roles in protection to toxic agents. Inhibition of

a protective protein like MTs by UV irradiation can be one of mechanisms through which UV causes lesions. In the present report, the inhibition of MT induction and cellular lesions by it were studied.

2. Research Method

Monochromatic radiation was obtained from a xenon lamp in combination with a monochromator. Cells of NB1RGB (normal human skin fibroblast), NCTC 2544 (human epitherial cells) or HeLa (human cervical carcinoma cell) were washed twice with phosphate-buffered saline (PBS) and irradiated in a ϕ35 mm dish in the presence of 1 ml PBS with a single dose of UV.

PBS in a dish was replaced by 2 ml of a cell culture medium containing a radioactive amino acid after the irradiation. Total proteins were labeled with 20 μC of ^{35}S-Methionine for 2 hours. MTs were labeled with 7 μC of ^{35}S-cystein for 18 hours. MTs were detected by fluorography of polyacrylamide gel (15 %) electrophoresis of carboxymethylated cell lysates.

Norther hybridization of MT-mRNA was performed employing a degoxigenin-labeled human MT-IIA probe.

Fig. 1

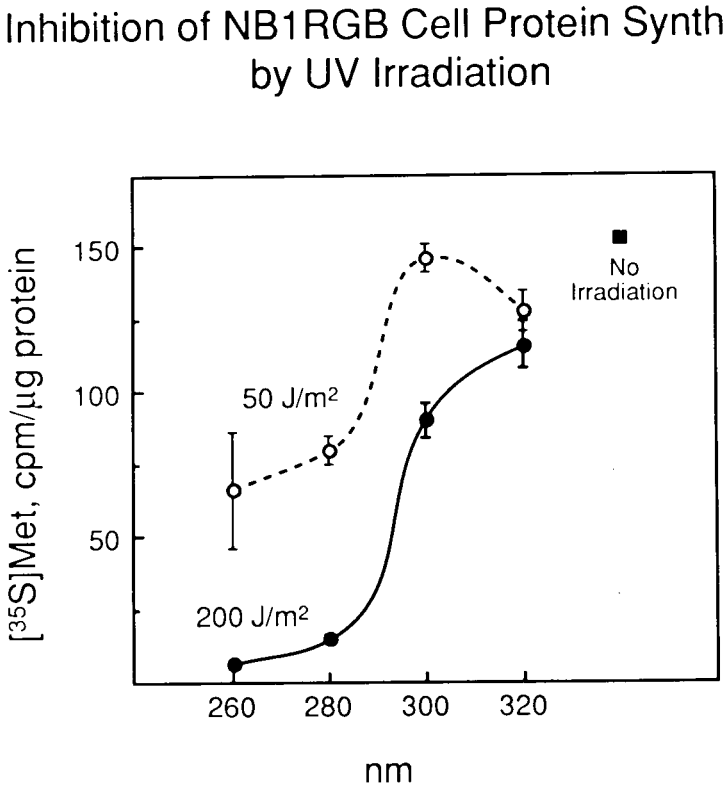


Fig. 2

Inhibition of MT Synthesis in NB1RGB Cells by UV Irradiation

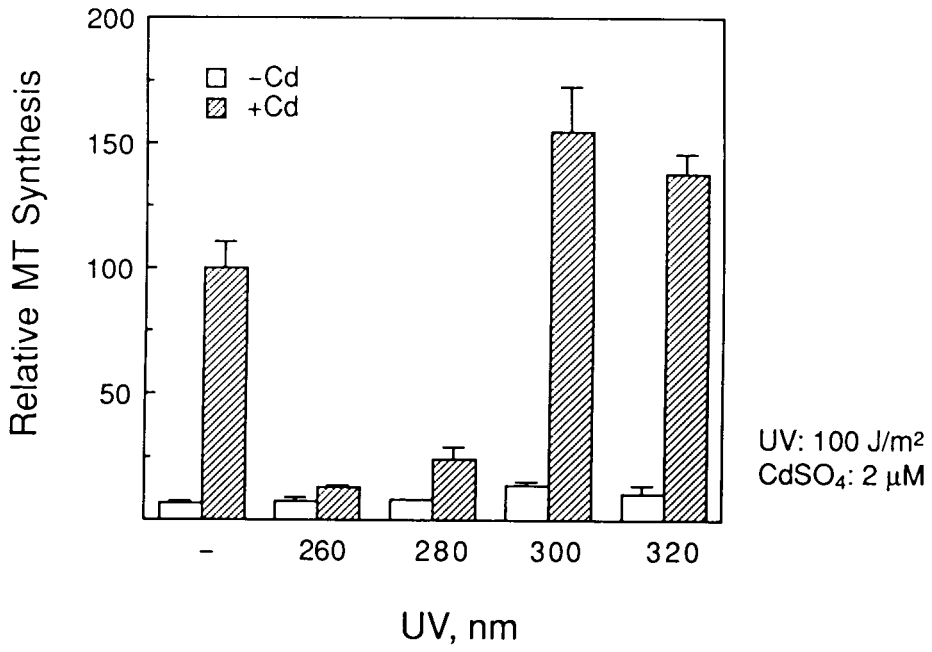


Fig. 3

Inhibition of Cd-, Zn- and Dexamethasone-induced MT Synthesis in NB1RGB Cells by UV_{280} Irradiation

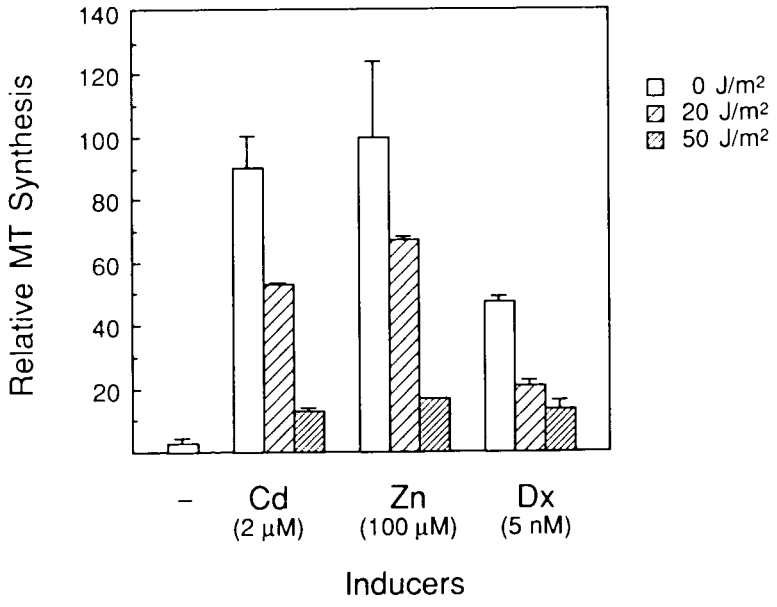


Fig. 4

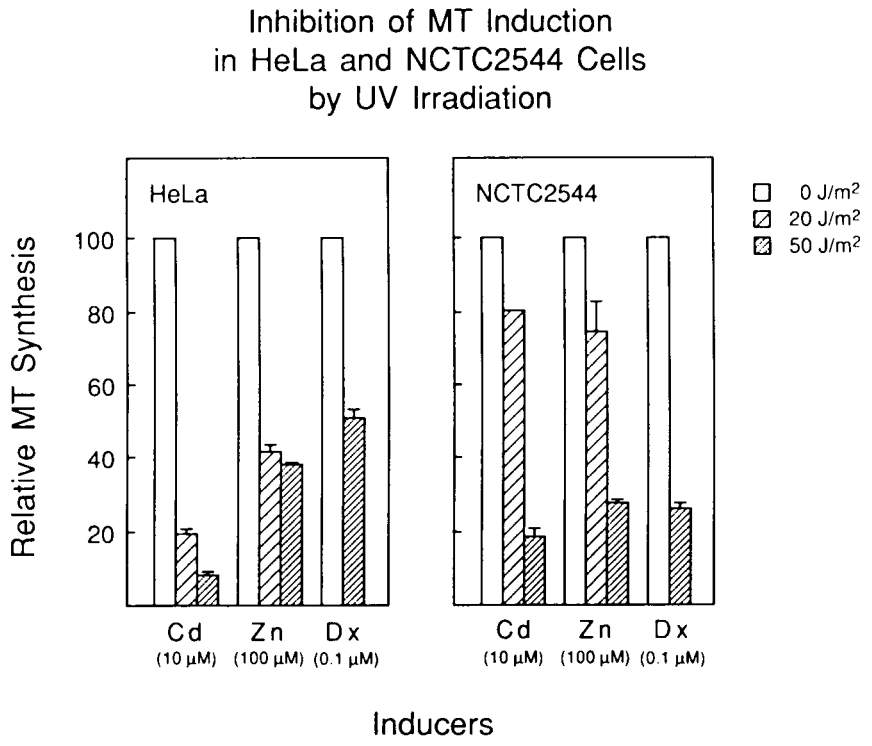

Inhibition of MT Induction
in HeLa and NCTC2544 Cells
by UV Irradiation
HeLa
NCTC2544
0 J/m²
20 J/m²
50 J/m²
Relative MT Synthesis
100
80
60
40
20
Cd (10 μM)
Zn (100 μM)
Dx (0.1 μM)
Inducers

Fig. 5

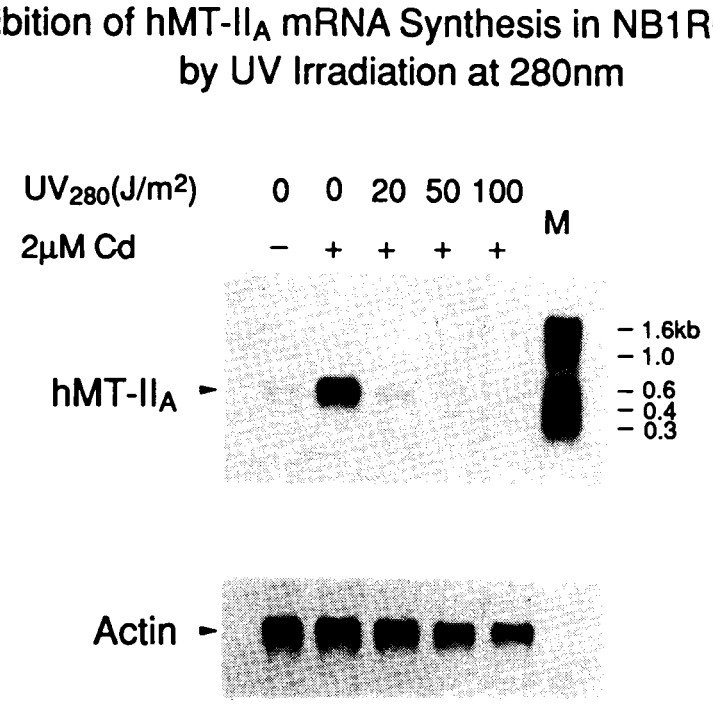
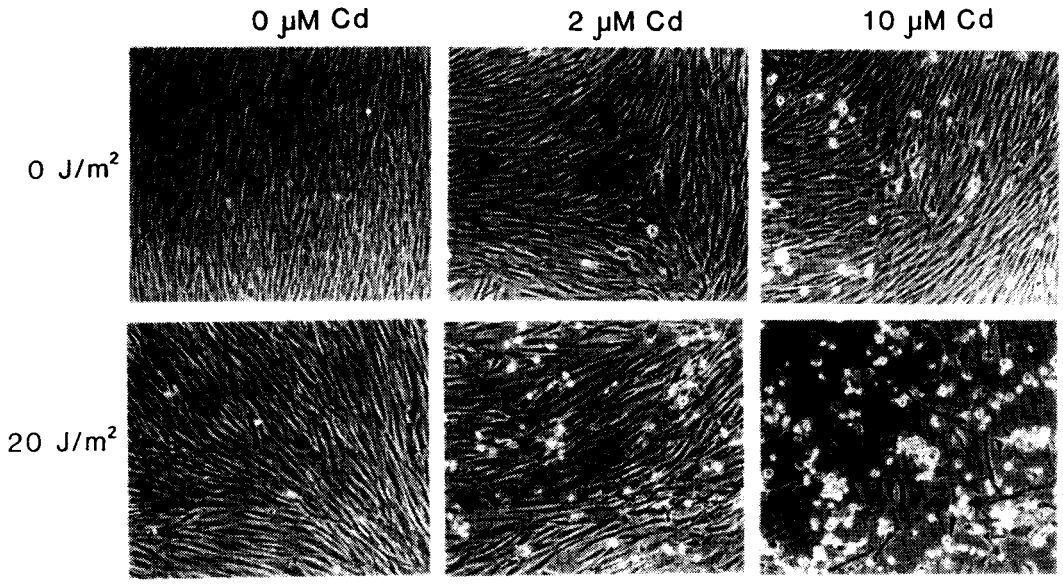

Inhibition of hMT-IIA mRNA Synthesis in NB1RGB Cells
by UV Irradiation at 280nm
UV280(J/m²) 0 0 20 50 100 M
2μM Cd − + + + +
hMT-IIA
Actin
1.6kb
1.0
0.6
0.4
0.3

Fig. 6



4. Results and Disscussion

Fig 1 shows effects of wavelength on protein synthesis in NB1RGB. UV at 260 nm and 280 nm inhibited protein synthesis much more than longer wavelength UVs. The same situation was observed in MT induction (Fig 2). These results caution us that data is not reliable when UV light with undefined wavelength is used. MT induction was inhibited as well by 280 nm irradiation when zinc or dezamethazone was used as an inducer (Fig 3).

The inhibitory effects of UV irradiation were also evident in NCTC 2544 and HeLa cells (Fig 4). The results suggest that the inhibition of MT induction caused by UV is common cellular response.

Northern hybridization in Fig 5 shows an increase in MT-mRNA level was repressed markedly after an addition of Cd in the 280 nm-irradiated cells. The inhibition of MT induction was achieved at transcriptional level.

It is likely that 280 nm-irradiated cells decrease resistance to heavy metal since MT induction is suppressed. Fig 6 shows this speculation is correct. Double exposures to UV and Cd were more hazardous than single exposures to each of them.

These results suggest that UV is not only perilous as short wavelength light but also toxic as a inhibitor which suppresses induction of protective proteins like MTs which are assumed to confer the resistance to toxic stimuli on cells.