A-4.4.2 Experimental Study on the Pathogenesis of Cataract caused by Ultraviolet Ray

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Abstract

The main objective of this study is to elucidate how lens lose its transparency to get opacity, which leads to cataract formation, due to continued exposure to ultraviolet ray B (UVB). In the present study, utilizing cultured organ and cell derived from bovine lens, we investigated the effects of UVB upon chrystallins, proteins unique to lens as well as gene expression of some oncogenes. We found that α- and β- chrystallins were subject to degradation and modification and speculated that the conformational change in α-chrystallins may lose its chaperon function and aggravate the lens opacity. In addition, we found that UVB irradiation caused an increased expression of c-fos and c-jun genes, single strand break of DNA chain, and induction of apoptosis of lens epithelial cells. These results explain a possible mechanism of opacification in vivo.

Key Words Cataract, Lens, Opacity, Ultraviolet ray

Introduction

It has been recognized that ultraviolet ray (UV) is a main cause of lens opacity and cataract. In our daily life, only UVA and B can be reached from the solar radiation, and the increase in UVB level due to depletion of ozone layer draw attentions from the public, administration, and the academic societies with regard to the possible increase UV-related health effects. The opacity of the lens depends upon the regularity of structural arrangement of thee lens epithelium and the harmonization of proteins in this tissue.

In the eye tissue, there are several protective machinary against irradiation [1]. The cornea can absorbs UVC (below 280 nm) but not UVA or B, both of which penetrate through the lens tissue. Since there is no vasculature system in the lens tissue, free radicals produced in the cells by UV irradiation exert oxidative stress to the cell and eventually causes lens opacity[2]. However, how UVB irradiation affects the structural integrity of the lens epithelium as well as protein structure remains to be studied. In the present study, we aimed at clarifying effects of UVB upon the cultured lens and primary cultured lens epithelial cells in terms of proteins and gene expression.

Materials and Methods

Preparation of lens and epithelial cells and UVB irradiation: Lens were removed from Wistar rats (6-7 weeks old) under pentobarbital anesthesia and used for organ culture studies.

The cultured lens was exposed at 5kJ/m2 for specified period. The lens epithelial cells were collected from bovine lens and used for primary cultured cell studies. They were exposed to UVB (312 nm, maximum) at a dose range from 10 to 400 J/m2.

Biochemical analyses: Glutathione level in the lens was determined by fluorescent labeling HPLC method. One and two dimensional SDS -PAGE and Western blotting analyses were carried out by the authentic methods. To determine the primary structure of purified proteins, appropriate spots of the 2-D SDS-PAGE were cut out and subjected to trypsin digestion, followed by Tricin-SDS-PAGE. After Western blotting to PVDF membrane, the appropriate PVDF spot was directly analyzed by the protein sequencer. Northern blot analysis was carried out for c-fos and c-jun mRNA. DNA strand break was detected by single cell assay. Apoptotic cells in the primary cultured epithelial cells were detected by Hoechist staining.

Results and Discussion

Lens opacification and alterations in reduced form of glutathione: UVB irradiation at a dose of 5 kJ/m2 caused opacification in the outer region of the lens cortex at hour 24 with a significant decrease (approximately 70% compared to non-irradiated control) in GSH level (Fig.1). At hour 72, the opacification aggravated not only in the cortex but also in the nucleus of the lens, with a further decrease in GSH level; 10% of the control level. In contrast, UVA irradiated lens showed a slight decrease in opacity and glutathione level just after irradiation (0-hr), but the lens showed transient recovery at hour 24 but aggravation by hour 72 in terms of transparency and GSH level. As shown in the present study, the concentration of reduced form of GSH reflects the transparency/opacity of the lens. It is considered that UVB irradiation is more harmful to the lens tissue compared to UVA at the same dosage depending upon their energy.

Alterations to soluble proteins: Since a pilot study showed that UVB irradiation affected protein structure more significantly at hour 72 rather than hour 24, we decided to use lens specimens at hour 72. SDS-PAGE analysis show that UVB irradiated lens specimens (Fig. 2a; lanes 4 and 5) do not have as much 30 kD protein as non-irradiated control lens specimens (Fig. 2a; lanes 1 and 2) but have a new 20 kD protein band (Fig. 2a; lanes 3 and 4). No significant change was observed in the UVA-irradiated lens specimens (Fig. 2a; lanes 6 and 7). Since the newly found 20 kD protein band reacted with anti- α B-chrystallin antibody by Western blotting analysis (Fig. 2b), the band was not a degradation products of low-molecular weight heat shock protein (sHSP) but that of α B-chrystallin.

To study further alterations of protein structures and possible appearance of newly induced proteins by UVB, we analyzed lens specimens by 2D SDS-PAGE. As shown in Fig. 3, UVB-irradiated lens specimens showed appearance of at least three protein spots on the gel, tentatively designated as A, B and C. After examining the primary structure of each of these bands after trypsin digestion, the spot A and B had the identical homology with β A4–chrystallin (residues no. 133-144) and α A2-chrystallin (residue no. 55-67), respectively. Because we could not analyze the primary structure of the spot C, we used Western blot analysis and found that the spot C reacted with anti- α B-chrystallin antibody. These proteins are considered to appear after UVB irradiation caused degradation and modification of α-chrystallins, which results in changes in isoelectric point and apparent molecular weight. The alteration of α-chrystallin structure in the lens may decrease its molecular chaperon function, subsequently aggregation of other proteins will not be rescued, and eventually account for the opacification of the lens.

Effects of UVB on oncogene expression: Since the growth of lens epithelial cells can be suppressed by UVB irradiation, the expression of oncogenes, c-fos and c-jun, was examined by Northern blot analysis. Their mRNA levels became highest between hr 0.5 to 1 at 100 J/m2 with a decrease thereafter. This time-course change is similar to the pattern of growth of the lens epithelial cells. These results may be related to the observation of the overexpression of the oncogenes in cultured cells exposed to hydrogen peroxide[3]. In the present experiment, we could not detect the induction of suppressor oncogene, p53 gene.

Detection of DNA strand break and apoptotic cells: We could not find DNA strand breaks in non-irradiated lens epithelial cells, but detected the strand breaks in UVB-irradiated cells. The degree of strand breaks was dependent upon the UVB dose. The cells exposed to 100 J/m2 showed some recovery at hour 48 after the irradiation. When we examined the nucleus of the lens epithelial cells, there were aggregation and fragmentation at hour 48 by 1 kJ/m2. Since we could find changes in cell shapes as found in apoptotic cells, we would conclude that the lens epithelial cells were also subjected to cell death by apotosis under the present condition.

Acknowledgement

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References

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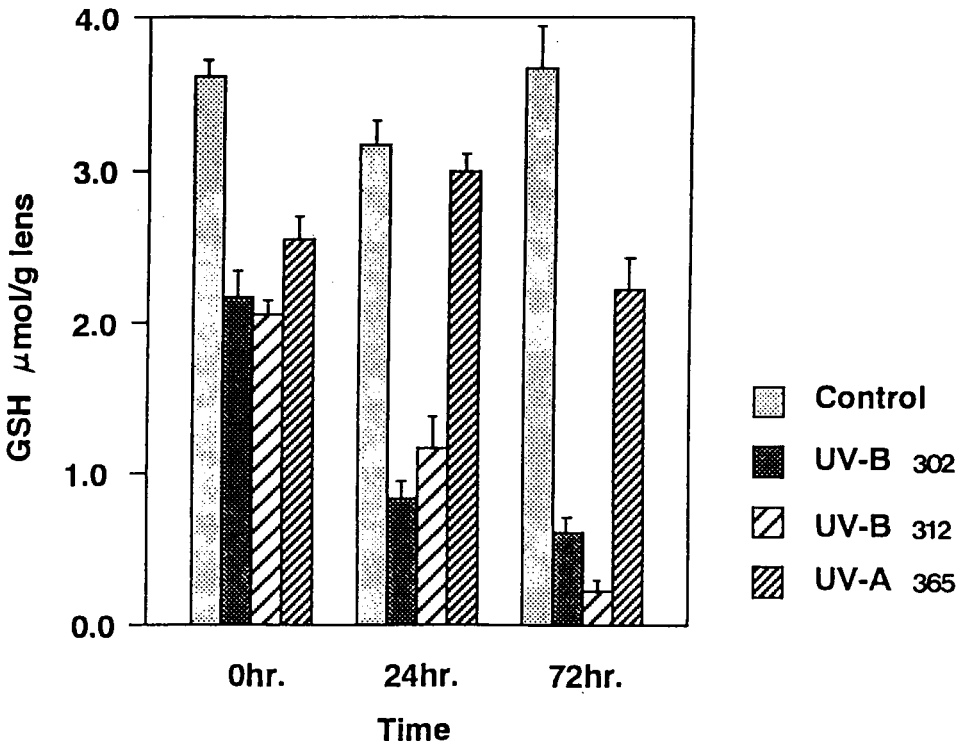
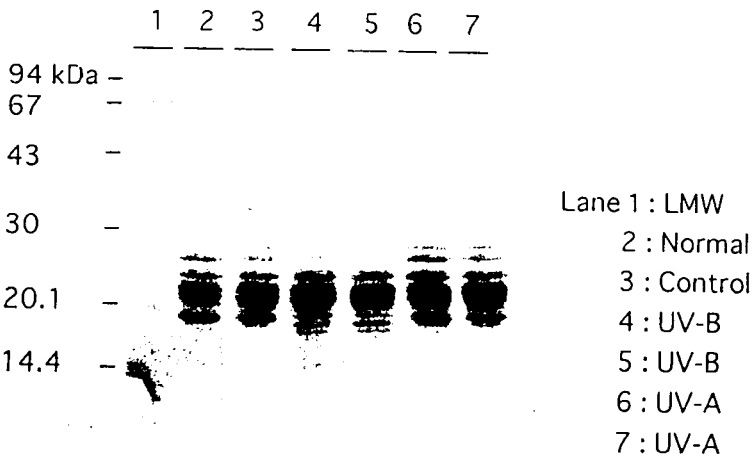


Fig. 1. Change in amounts of reduced form of glutathione by UVA and B irradiation.

(a) SDS-PAGE.



(b) Western blotting.

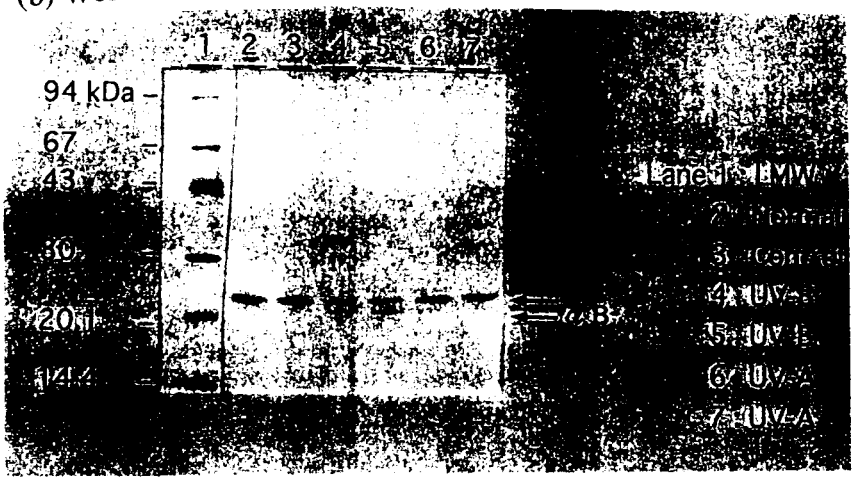


Fig. 2. SDS-PAGE and Western blot analyses of the lens soluble proteins upon UVB irradiation. (a) SDS-PAGE. (b) Western blotting.

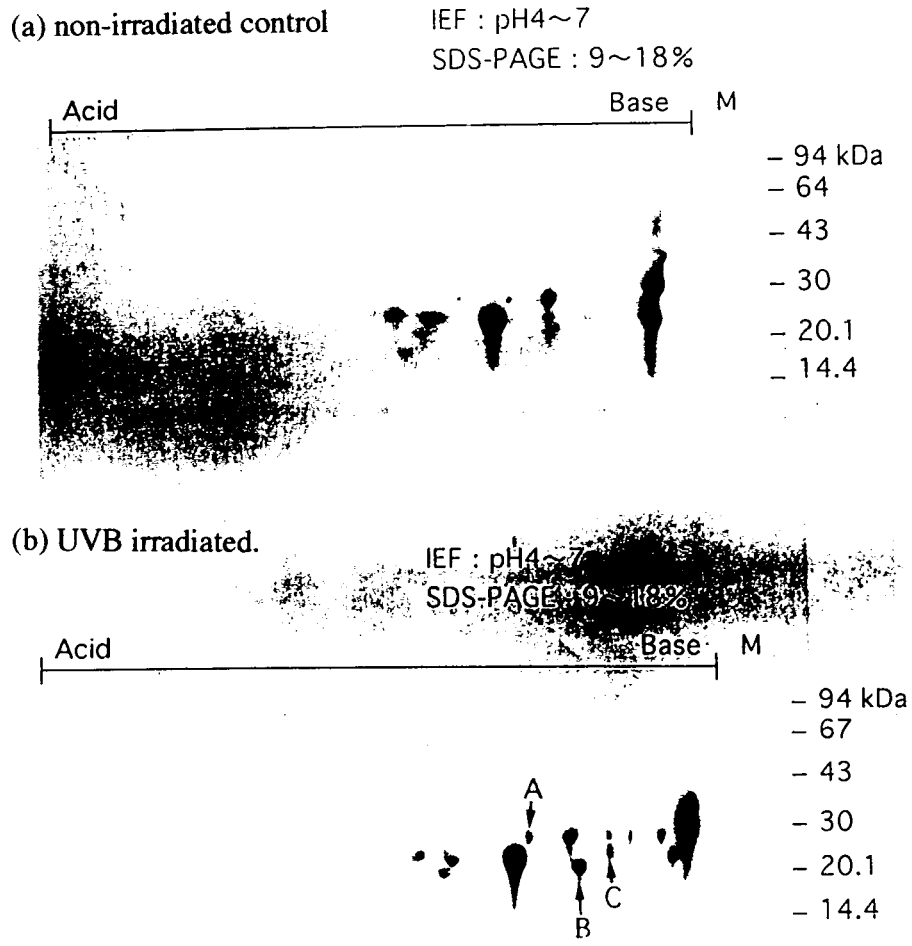


Fig. 3. Two dimensional electrophoretic analysis of acidic protein fraction of the lens proteins.
(a) non-irradiated. (b) UVB irradiated.