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Comparison of hair shaft damage after UVA and UVB irradiation

SOO-YOUNG JEON, LONG QUAN PI, and WON-SOO LEE,
Department of Dermatology and Institute of Hair and Cosmetic Medicine, Yonsei University Wonju College of Medicine, Ilsan-dong 162, Wonju, Korea.

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Synopsis

Sunlight, especially ultraviolet (UV) light-induced hair damage is difficult to avoid during daily life. Concerns about the effects of ultraviolet light on hair are emerging recently. These photochemical changes mainly come from damage to hair proteins and melanins. In this study, we performed experiments to find the patterns of morphological and biochemical changes in UV-light-induced damage to hair by scanning and transmission electron microscopy and hair protein analysis. In our results, morphological damage is significant in UVB-irradiated hairs, while biochemical changes are greater in UVA-irradiated hairs.

INTRODUCTION

Changes in hair fibers induced by UV light are largely composed of physical and chemical changes. As for physical changes, dryness, reduced strength, rough surface texture, loss of color, decreased luster, stiffness, and brittleness may occur. In respect to chemical changes, hair proteins, lipids, and hair pigments can change.

When light irradiates the hair shaft, the amino acids in the cuticle are altered more than those in the cortex because the outer layers receive higher intensities of radiation (1). Beyak *et al.* (2) reported that sunlight and UV light decreased tensile properties of wet human hairs and that degrees of damage are related to the total doses of radiation rather than a specific wavelength. But, in another study, it was reported that the specific wavelength of UV irradiation, especially between 254 and 400 nm, seemed to damage hair more severely than did other wavelengths (3). Hoting *et al.* (4) showed that the cell membrane complex (CMC) lipids of hair fibers was degraded mainly by visible light but that UVA and UVB light also participated in the damaging process. He explained that weakening of the CMC and multiple fractures in hair occurred by a combination of the effects of visible and UV light.

Address all correspondence to Won-Soo Lee.

There are several amino acids that are brittle to light; these are cystine and methionine among the sulfur-containing amino acids, phenylalanine and tryptophan among the aromatic and ring amino acids (which are associated with photo-yellowing of hairs), and histidine and proline (5,6). A gradual increase in brittleness and a loss of structural differentiation come from the breakdown of disulfide bonds within the structural units of the A-layer, exocuticle, and matrix. In the restoration process, new intramolecular and intermolecular crosslinks occur through the reaction of carbonyl groups (1). In this study, we carried out morphological evaluation and protein analysis of the hair shaft after UV irradiation, comparing the pattern of damage by UV light with different wavelengths.

EXPERIMENTAL

For this study, a healthy 28-year-old Korean male was selected whose hair was in good condition. He had not used hair care products excessively nor experienced any factors known for inducing hair injuries over the previous six months. Hair samples were collected and irradiated to various doses of UVA (maximum 6,680J) and UVB (maximum 58,320mJ) using the HOUVA II UAB-001 phototherapy system (National Biological Corporation, U.S.A.). The distance from the lamps to the hair shafts was 50 cm, the relative humidity was 30%, and an external cooling device with cold water and ice was applied to minimize the heating effect. After irradiation, we accomplished morphological evaluation by scanning electron microscopy, conventional transmission electron microscopy, and lipid transmission electron microscopy using Lee's fixative (0.5% RuO_4 : 2% OsO_4 : 0.2 M cacodylate buffer = 1 : 1 : 1) (7). Concurrently, we performed labile hair protein analysis according to the method of Inoue *et al.* (8), who have reported the transformation patterns of hair protein after permanent waving and hair dyeing *in vitro.* According to them, stable protein portions in normal hair are transformed to labile protein, the internally formed soluble protein, and the major components of the labile protein are ubiquitins (8). We proposed that this phenomenon would happen in irradiated hair shafts. The results are summarized as follows.

RESULTS AND DISCUSSION

MORPHOLOGICAL FINDINGS USING ELECTRON MICROSCOPY

Scanning electron microscopic study

(a) Before UV irradiation: Normal hair cuticles look undamaged and have intact, tightly overlapping cuticular scales with smooth, homogenous, and shiny surfaces (Figure 1A).
(b) After UVA irradiation: Focal lifts at edges of the cuticles, focal losses of cuticular edges, and generalized irregular contours of cuticle cells are observed (Figure 1B).
(c) After UVB irradiation: Focal losses of cuticular edges, focal cuticular lifts at the edges of the cuticles, and focal cuticular detachments appear (Figure 1C). In comparison to findings after UVA irradiation, more severe cuticular damage seems to occur in UVB-irradiated hair shafts.

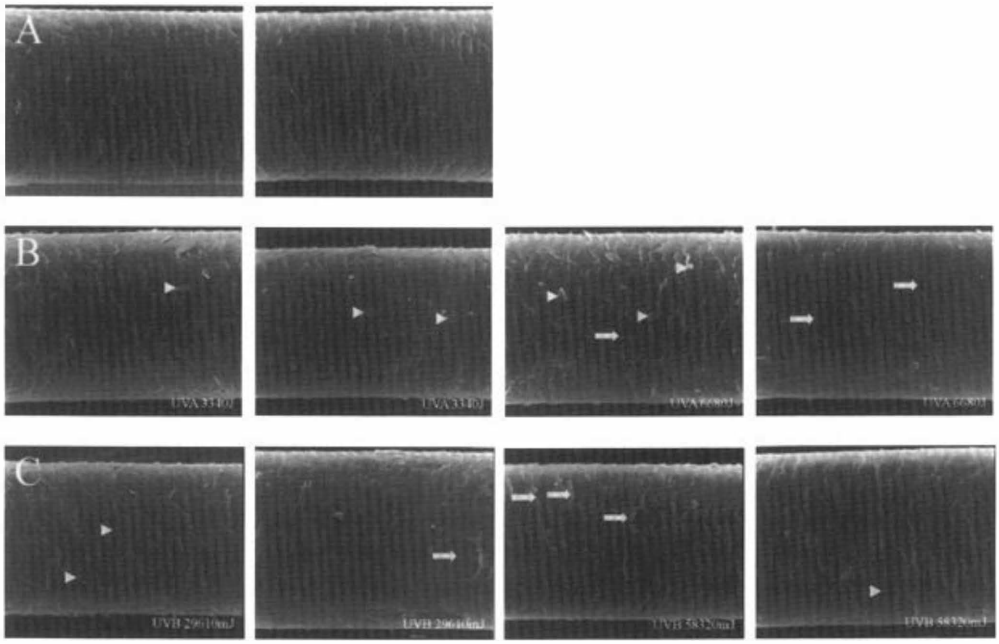

Figure 1. SEM findings in hair shafts: (A) Before irradiation. (B) After UVA irradiation. (C) After UVB irradiation. Arrow: focal lifts at edges of cuticles. Arrowhead: focal losses of cuticular edges.

Conventional transmission electron microscopic study

(a) Before UV irradiation: The hair shows concentric arrangements of smoothly bounded cuticular cells containing the normal complement and distribution of the A-layer, exocuticle, and endocuticle subcomponents (Figure 2A).
(b) After UVA irradiation: Variable-sized holes in the endocuticles, cleavage along the endocuticles by confluence of the holes, and cuticular detachment appear (Figure 2B). The damage is more severe after high-dose irradiation of UVA.
(c) After UVB irradiation: Similar damage is observed (Figure 2C), but in comparison to findings after UVA irradiation, cleavage along the endocuticle and cuticular detachment is more severe. There remain only two to three cuticular layers in several parts, due to extensive damage.

Lipid transmission electron microscopic study with special fixative

(a) Before UV irradiation: There are intact intercellular lipid layers (Figure 3A).
(b) After UVA irradiation: There are some bulging portions in the intercellular lipid layers and small focal lacunae along the intercellular spaces (Figure 3B).
(c) After UVB irradiation: Several bulging portions in the intercellular lipid layers are also observed (Figure 3C).

BIOCHEMICAL FINDINGS WITH PROTEIN ANALYSIS

Figure 4 shows a western blot analysis of UV-light-irradiated hair shafts with polyclonal ubiquitin antibodies according to the laboratory method of Inoue *et al.* (8). There are continuous positive findings around the 10 kDa area after UVA irradiation. In com-

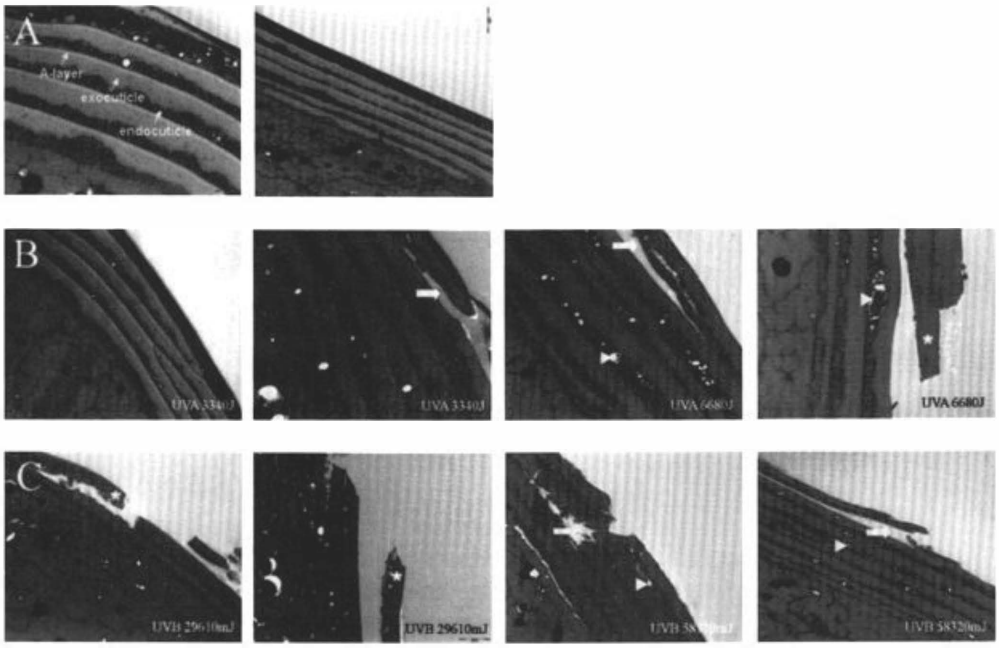

Figure 2. Conventional TEM findings in hair shafts: (A) Before irradiation. (B) After UVA irradiation. (C) After UVB irradiation. Arrowhead: holes in the endocuticles. Arrow: cleavage along the endocuticles by confluence of holes. Asterisk: confluence of holes.

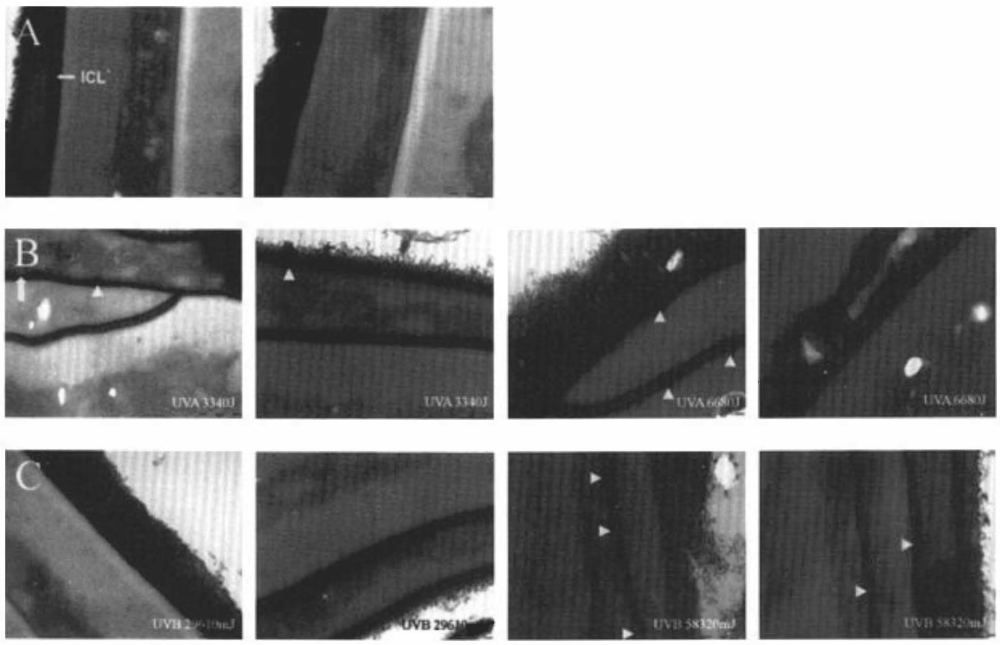

Figure 3. Lipid TEM findings in hair shafts: (A) Before irradiation. (B) After UVA irradiation. (C) After UVB irradiation. Arrowhead: bulging portions in the intercellular lipid layers. Arrow: small focal lacunae along the intercellular spaces.

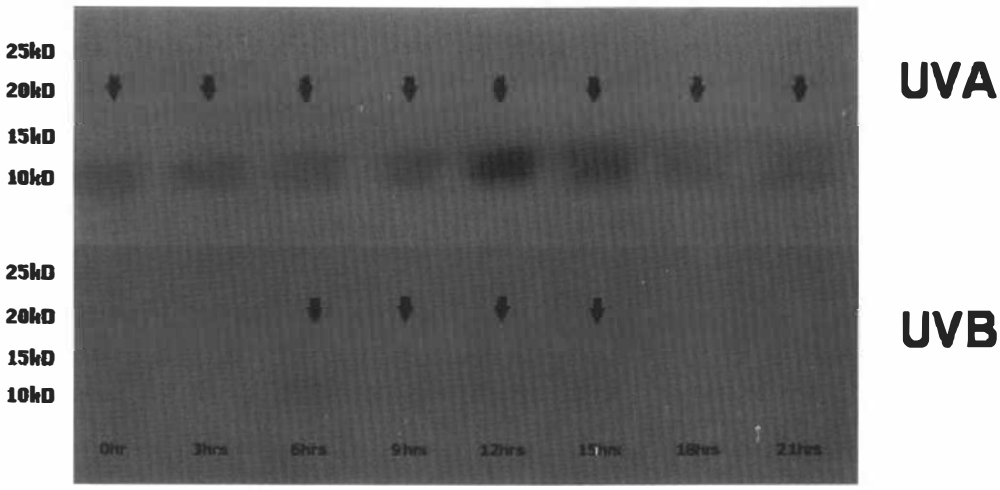

Figure 4. Western blot analysis of UV-light-irradiated hair samples with ubiquitin antibodies. Upper panel: after UVA irradiation. Lower panel: after UVB irradiation.

parison to the hair shafts after UVA irradiation, there are a few relatively weak positive findings around the 10kDa area after UVB irradiation. We think that the positive finding before UVA irradiation may result from the preceding natural weathering processes.

There have been several reports on the evaluation of photoaging and photoprotection of hair fibers with microscopy. Braida *et al.* (9) reported TEM findings on hair shafts after using two kinds of sunlight stimulators. On the other hand, Bousquet *et al.* (10) reported analysis of patterns of cuticles for evaluation of hair photoprotection with confocal microscopy. The former revealed the difference in melanin particles in irradiated hair samples with TEM, and the latter reported morphological findings in hair cuticles with confocal microscopy. In this study, we carried out investigations with four detailed tools having different strong points: SEM findings reveal superficial cuticular changes, conventional TEM findings reveal deep cuticular changes, and TEM findings using Lee's fixative reveal intercellular lipid layer changes more precisely. We also performed a protein analysis using ubiquitin antibodies. Ubiquitin-mediated degradations of regulatory proteins are known to play important roles in numerous processes, including cell-cycle progression, signal transduction, transcriptional regulation, receptor down-regulation, and endocytosis (11). In a recent study, Inoue *et al.* have suggested that ubiquitin could be an indicator of hair damage.

It was reported that the main wavelength of UV light absorbed by keratin was UVB, which could do damage mainly by the cleaving of the protein bonds and protein crosslinking (12). In the hair shaft, UVB is known to penetrate about 5 μm in depth, and the intensity decreases exponentially after that (12). Considering that intact hair cuticles are 6–10 layers, each with a thickness of 0.3–0.5μm, UVB seems mainly to affect the cuticles. This suggests that hair damage by UVB irradiation is mainly confined to the superficial layers of the hair shaft, the cuticle layers. We think our findings are related to the penetration depth of UV light mentioned above. UVA irradiation can penetrate deeply into the cortex, and so biochemical changes, including cuticles and

cortex together, may appear greater after UVA irradiation. On the other hand, UVB causes severe morphological damage, confined to the hair cuticles because of its restricted depth of penetration.

CONCLUSIONS

In summary, our morphological study shows relatively more destructive cuticular changes after UVB irradiation than after UVA, while disruptions of the intercellular lipid layer show similar results between UVA and UVB irradiation. However, in labile protein analysis, damaged labile hair proteins are much more observed after UVA irradiation than after UVB irradiation. Because high doses of UV light were irradiated to observe patterns of damage in this report, we'd like to plan to observe chronically photodamaged hair shafts as similar as possible to those in daily life. Based on the principal findings here, we hope it will be useful to study the photoaging of hair and photoprotective methods in hair. Other biochemical methods, including amino acid analysis and lipid analysis, would be helpful to understand the process of UV-light-induced damage to hair.

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