

# The Ultraviolet B Protection Effects of Topically Applied Melanosomes onto Human Skin

Sungbin Im<sup>1</sup>, Sungnack Lee<sup>2</sup>, Seung Kyung Hann<sup>1</sup> and Yoon-Kee Park<sup>1</sup>

*Melanosome is a cellular organelle that is composed of a melanosomal matrix and a brown biochrome, melanin which is formed by tyrosine-tyrosinase reactions. The melanosome is formed within the melanocyte and transferred to the surrounding keratinocytes through dendritic processes.*

*Human skin color is related to the number, size, type and distribution of melanosomes, and the major role of melanosomes is to prevent skin from injurious nonionizing ultraviolet radiation.*

*Controlled NaOH hydrolysis and centrifugation of human hair make it possible to isolate large amounts of melanosomes which are synthesized within the follicular melanocytes and transferred to hair matrix cells.*

*In this study, the sun protection factors of topically applied melanosomes isolated from human hair were evaluated using ultraviolet B phototesting. Topically applied melanosomes increased the minimal erythral doses. And the sun protection factors of each 50% and 25% melanosomal preparation were  $12.3 \pm 5.5$  and  $3.1 \pm 1.3$  respectively, and these ultraviolet B protection effects showed statistically significant differences from 10%, 5% and 1% melanosomal preparations and vehicle.*

*Form these results, the dose-related photoprotective role of melanosomes was confirmed.*

---

**Key Words:** Isolation of melanosomes, UVB protection effects

Human skin color is related to the number, size, type and distribution of cytoplasmic pigment particles called melanosomes, which contain a brown biochrome, melanin.

Melanin has an important role in preventing the skin damage from nonionizing ultraviolet radiation (Blios, 1966). The racial differences of skin color and response to ultraviolet radiation are dependent on melanosomes (Hashimoto et al. 1979). Dark black skin has a minimal erythral dose (MED) 33 times that of the Caucasian. The black skin also contains the greatest density of pigment, and has the largest melanosomes, all singly dispersed (Olson

et al. 1973). Black skin has more melanin in the epidermis than in white skin, thus approximately five times less UVB and UVA reach the dermis of blacks as compared with whites (Kaidbey et al. 1979).

Melanosomes are intracellular organelles which are formed by melanocytes and transferred to keratinocytes, and thus provide the protective role to ultraviolet radiation.

To investigate the photoprotective role of melanized melanosomes, we isolated these organelles from human black hair and applied them at varying concentrations onto the skin of human volunteers to determine their protective factors against UVB radiation.

## MATERIALS AND METHODS

### Materials

Black hairs from Korean males in their twenties were collected as a source of melanosomes.

### Isolation of melanosomes

Hairs were washed with cold acetone for 10 min-

---

Received October 29, 1991

Accepted January 14, 1992

Department of Dermatology<sup>1</sup>, Yonsei University College of Medicine, Seoul, Korea

Department of Dermatology<sup>2</sup>, College of Medicine Ajou University, Suwon, Korea

This study was supported by a Department project Grant from Yonsei University College of Medicine(1990)

Address reprint requests to Dr. S Im, Department of Dermatology, Yonsei University College of Medicine C.P.O. Box 8044, Seoul, Korea, 120-752

utes and diethyl ether for 10 minutes and dried in room air. These were treated with 1 N NaOH for 15 hours at 37°C in a shaking incubator. The suspension was centrifuged at 2,500 × g for 10 minutes. The first supernatant was centrifuged at 3,500 × g for 10 minutes. The second supernatant was centrifuged at 7,800 × g for 10 minutes. The precipitate was suspended with normal saline, and this suspension was centrifuged at 7,800 × g for 10 minutes. The precipitate was melanosomes. All centrifugation procedures were performed at 0°C (Im *et al.* 1991).

#### Electron microscopic examinations of melanosomes

For transmission electron microscopy, the diluted pellet of melanosomes was prefixed in a mixed solution of 2.5% glutaraldehyde and 1% paraformaldehyde and postfixed with osmium tetroxide. After dehydration with graded ethanols, the sample was embedded in Epon, cut into several ultrathin sections, and stained with uranyl acetate followed by lead citrate. The sections were examined by a Hitachi H-500 electron microscope.

For scanning electron microscopy, the diluted pellet of melanosomes was prefixed in a mixed solution of 1% glutaraldehyde and 1% paraformaldehyde. The prefixed melanosomes were filtered through a 0.4 μm nucleopore membrane filter. The filtered melanosomes were arrayed in a single layer and washed with phosphate buffer (pH 7.4), followed by 1% osmium tetroxide. Following osmiation, the sample was dehydrated through graded ethanols, transferred to isoamyl acetate, dried with a critical point drier (Hitachi ICP2), mounted on stubs, coated with gold in a sputter coater, and viewed through a Hitachi H-450 scanning electron microscope operated at 20 kV.

#### Preparation of topically applied melanosomes

Isolated melanosomes by differential centrifugations were mixed with hydrophilic ointment base in the concentration of 50%, 25%, 10%, 5%, 1% (weight/weight).

#### Phototesting

Phototesting for the MED of ultraviolet light in its B range was performed with a bank of fluorescent lamps (FS 36 T12-UVB-HO lamp, Elder Co., Ohio). The irradiance of light source was measured with appropriate radiometer and probe (IL 442 Radiometer, International Light Inc., Georgia). For determi-

nation of the protective effect of melanosomes, the above melanosomal preparations and hydrophilic ointment base, 8% homomenthyl salicylate (E. Merk, Darmstadt, Germany) as an internal standard were applied onto the back skin of 12 healthy Korean males in their twenties who did not have sun exposures at least for 4 months. They were all applied to the same subjects at the rate of 2 μl/cm<sup>2</sup> and were spread carefully to ensure complete, uniform coverage at the test sites.

Treated sites were then permitted to air dry at least 15 minutes before proceeding to the next step. The untreated, normal test site (1 cm<sup>2</sup>) was irradiated by graded increments of UVB doses; 10, 20, 30, 40, 50, 60, 70, and 80 (mJ/cm<sup>2</sup>). The treated test sites (1 cm<sup>2</sup>) were also irradiated by graded increments of UVB doses; 60, 90, 120, 150, 200, 400, 600, and 800 (mJ/cm<sup>2</sup>).

The MED is defined as the smallest ultraviolet B dose that produces minimally perceptible erythema approximately 24 hours after ultraviolet exposure. The MED of untreated and treated test sites was evaluated and the sun protection factor (SPF) of each preparation was calculated as follows;

$$\text{SPF} = \frac{\text{MED of treated skin}}{\text{MED of untreated skin}}$$

#### Statistical analysis

Resultant SPF was expressed as mean ± standard deviation and using the analysis of variance (ANOVA), the statistical significance was determined.

## RESULTS

On the transmission electron microscopic examination, the isolated melanosomes showed a lower degree of degradation and less contamination of keratins (Fig. 1).

On the scanning electron microscopic examination, the global structures of melanosomes with variable sizes and shapes could be observed (Fig. 2).

The MED of the Korean young male was 54.2 ± 13.8 mJ/cm<sup>2</sup>. The erythematous responses of the application site of the above melanosomal preparations were variable according to the concentration of the melanosomes. The preparation of 50% melanosomes in hydrophilic ointment base appeared black in color and represented SPF values of 12.3 ± 5.5. The SPF of 25% melanosomal preparation revealed 3.1 ± 1.3. These values showed statistically significant

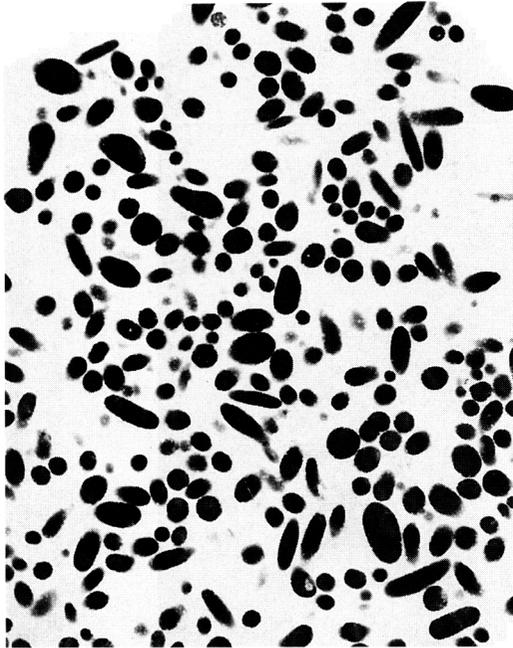


Fig. 1. Transmission electron microscopic findings of isolated melanosomes. Lower degradation of melanosomes or less contamination of keratins are noted ( $\times 12,200$ ).

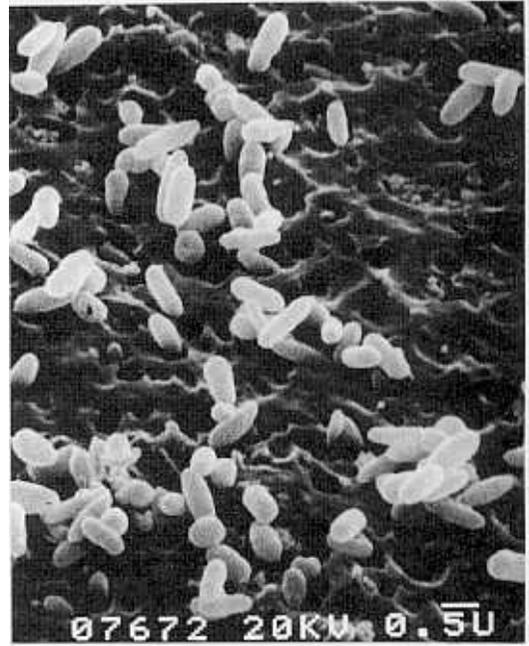


Fig. 2. Scanning electron microscopic findings of isolated melanosomes. The global structures of melanosomes are observed ( $\times 14,000$ ).

Table 1. Evaluations of minimal erythral doses (MED) and sun protection factors (SPF) 24 hours after ultraviolet B exposure

Subject	MED (mj/cm <sup>2</sup> )				SPF			
	Normal skin	50%M	25%M	10%M	5%M	1%M	H-base	8%HMS
	30	20		2			2	3
	40	15		2.3			2.3	5
	70	2.9		1.3			1.7	2.9
4	40	20	1	3.8	3.8	2.3	2.3	3.8
5	50	16	3	2.4	1.8	1.8	1.8	4
6	50	4	3	2.4	1.8	1.8	1.8	4
7	70	8.6	2.1	1.3	1.3	1.3	0.9	5.7
8	50	16	1.8	1.2	1.2	1.2	1.2	4
9	60	10	2	1	1	1	1	3.3
10	70	11.4	5.7	2.1	1.3	1.3	1.3	2.9
11	70	11.4	2.9	2.1	2.1	1.7	1.3	2.9
12	50	12	4	1.2	1.2	1.2	1.2	4
Mean	54.2	12.3	3.1	1.9	1.7	1.5	1.5	3.8
SD	13.8	5.5	1.3	0.7	0.8	0.4	0.5	0.9
N	12	12	9	12	9	9	12	12

M : melanosomes in H-base (w/w)  
 H-base : hydrophilic ointment base  
 HMS : homomenthyl salicylate  
 SD : standard deviation  
 N : number of subjects

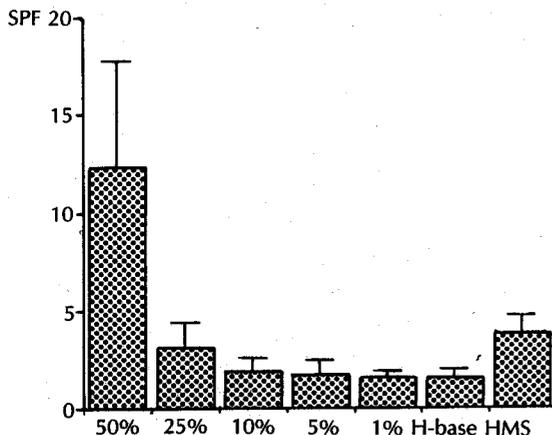


Fig. 3. SPF values for each preparation.

M : melanosomes in H-base (w/w)  
 H-base: hydrophilic ointment base  
 HMS : homomenthyl salicylate  
 SD : standard deviation  
 N : number of subjects

UVB protection than the hydrophilic ointment base ( $P < 0.05$ ). The 10%, 5% and 1% melanosomal preparations showed less degree of UVB protection which was not statistically significant. The SPF of the internal standard (8% homomenthyl salicylate) in this study was  $3.8 \pm 0.9$  and the reproducibility of this test was assured (Table 1, Fig. 3).

## DISCUSSION

Melanosomes and melanin are the major determinants of skin color among individuals and the major sun-protecting agents of skin from nonionizing ultraviolet radiation (Bliss, 1966).

The disorders which are related to the melanocytes, melanosomes, and melanin are albinism (King and Witkop, 1976), vitiligo (Lerner, 1971), Ota's nevi (Fitzpatrick, 1981), nevocellular nevi (Eady et al., 1977) and malignant melanoma (Flerning et al. 1975).

The important role of melanosomes in the sun-related skin disorders such as sunburn, freckles, lentigines (Weiss and Zelickson, 1977), and skin aging is now a primary focus of interests for dermatologists.

Photoreactions involving photosynthesis, vision, vitamin D synthesis, killing of pathogen, photother-

apy, and photochemotherapy (Giese, 1976) are considered good and beneficial, but the sunburn, skin cancer, phototoxic and photoallergic reactions (Ramsay and Obershkova, 1974) are recognized as harmful. These harmful effects of ultraviolet radiation depend upon the length and the frequency of exposure, the intensity and the wavelength of sun light, and the skin type of the individual.

The solar radiation spectrum which affects the human skin at the earth's surface is ultraviolet A (320~400 nm) and ultraviolet B (290~320 nm). Ultraviolet A induces skin tanning and ultraviolet B induces erythematous skin response. Thus damage of skin is mostly dependent on the ultraviolet B radiation (Pathak, 1982). But the skin itself has naturally produced defense mechanism for ultraviolet radiation including changes of distribution of melanosomes and new melanogenesis (Parrish, 1982). The photoprotective role of melanosomes and melanin is related to one or more of the physical and biochemical properties; scattering and degrading radiation to heat, absorption and immediate oxidation of radiation, and quenching of free radicals (Pathak and Fitzpatrick, 1974).

The concept of SPF was originally proposed by Greiter (1974), and this has been a valuable parameter of sunscreens. The SPF of the melanized skin depends upon the skin type from 1.0 (skin type I) to 5.0 (skin type VI) (Pathak and Fenselow, 1983). Dark black skin has a MED 33 times that of the Caucasian, contains the greatest density of pigment, and has the largest melanosomes, all singly dispersed (Olson et al. 1973). Consequently, approximately five times less UVB and UVA reach the dermis of blacks as compared with whites (Kaidbey et al. 1979). In contrast to this, the skin of vitiligo and albinism is more sensitive to sunburn. These responses are determined according to melanin and melanosomes.

Melanosomes are the small organelle which are dispersed in the melanocytes and keratinocytes. However, the isolation of melanosomes from the keratinous structures is difficult because of the resistance of keratin to chemical agents (Filson and Hope, 1957; Borovansky and Hach, 1972). Borovansky and Hach (1986) performed very excellent isolation of melanosomes without causing severe structural damage to the melanosomal protein. By modification of the methods of Borovansky and Hach (1986), we isolated the large amount of melanosomes from human hair with minimal contamination and degradation (Im et al. 1991).

Electron microscopy is the best means for the

evaluation of the isolated melanosomes. Transmission electron microscopic examination is useful in observing the internal structures of melanosomes. However, it is not adequate to evaluate the global morphology of melanosomes because it exhibits variable features determined by the direction of the cross-section. In this study, using the scanning electron microscope, the global structure of melanosomes and the size variations could be observed.

With the utility of large amount of pure and less degraded melanosomes, we could performed the direct photoprotective effect of melanosomes in vivo. The 50% and 25% melanosomal preparations showed remarkable concentration-related UVB protection.

These results are in accord with the skin type-related ultraviolet protection effects, and the photoprotective role of melanosomes are confirmed. But it remains unclear whether the melanin or the melanosomal protein alone are in contribution to the photoprotective effects.

Through this study, the dose-related photoprotective role of melanosomes was confirmed.

## REFERENCES

- Blios MS: On the spectroscopic properties of some natural melanins. *J Invest Dermatol* 47: 162-166, 1966
- Bolt AG: Interactions between human melanoprotein and chlorpromazine derivatives I. Isolation and purification of human melanoprotein from hair and melanoma tissue. *Life Sci* 6: 1277-1283, 1967
- Borovansky J, Hach P: Isolation of melanosomes from keratinous material—a new method. *Dermatologica* 145: 37-41, 1972
- Borovansky J, Hach P: Isolation of melanosomes from keratinous structures—current state of art. *Arch Dermatol Res* 279: 54-58, 1986
- Eady RAJ, Gilkes JIH, Wilson JE: Eruptive nevi. *Br J Dermatol* 197: 267-278, 1977
- Filson A, Hope J: Isolation of melanin granules. *Nature* 179: 221-224, 1975
- Fitzpatrick TB: *Classification of dermal pigmentation—blue skin*. In *Biology and Diseases of Dermal Pigmentation*. TB Fitzpatrick et al ed., Univ of Tokyo Press, Tokyo 1981, 65
- Fleming ID, Barnawell JR, Burlison PE, Rankin JS: Skin cancer in black patients. *Cancer* 35: 600-605, 1975
- Giese AC: *The sun, sun myths and sun worship*. In *Living with Our Sun's Ultraviolet Rays*. A.C. Giese et al ed., Plenum Press, New York, 1976, 1
- Greiter F: Sonnenschutzfaktor-Entstehung. *Methodik Perfumerie und Kosmetik* 55: 70-74, 1974
- Hashimoto K, Kohda H, Kumakiri M, Blender SLK Willis I: Psoralen-UVA-treated psoriatic lesions. *Arch Dermatol* 114: 711-722, 1979
- Im S, Lee S, Hahn K: Isolation and identification of melanosomes from human hair: A new approach to morphologic evaluation. *Annals of Dermatology* 3: 12-14, 1991
- Kaidbey KH, Agin PP, Sayre RM, Kligman AM: Photoprotection by melanin—a comparison of black and Caucasian skin. *J Am Acad Dermatol* 1: 249-260, 1979
- King RA, Witkop WJr: Hairbulb tyrosinase activity in acute cutaneous albinism. *Nature* 263: 69-71, 1976
- Lerner AB: *Natural control of pigment cells*. In *Biology of Normal and Abnormal Melanocytes*. T Kowamura et al., University Park Press, Tokyo, 1971, 3
- Olson PL, Gaylor G, Everett MA: Skin color, melanin and erythema. *Arch Dermatol* 108: 541-544, 1973
- Parrish JA: The scope of photomedicine. In *Science of Photomedicine*. JD Regan et al ed., Plenum Press, New York 1982, 3
- Pathak MA, Fitzpatrick TB: *The role of natural protective agents in human skin*. In *Sunlight and Man*. Pathak MA et al ed., Univ of Tokyo Presse, Tokyo, 1974, 25
- Pathak MA: Sunscreen: topical and systemic approaches for protection of solar radiation. *J Am Acad Dermatol* 1: 285-312, 1982
- Pathak MA, Fenselow DL: Photobiology of melanin pigmentation: Does/response of skin to sunlight and its contents. *J Am Acad Dermatol* 9: 724-733, 1983
- Ramsay CA, Obershokva E: Photosensitivity from nalidixic acid. *Br J Dermatol* 91: 523-528, 1974
- Weiss WL, Zelickson AS: Giant melanosomes in multiple lentiginos syndrome. *Arch Dermatol* 113: 491-494, 1977