cosmetics

MDPI

Article

New Method of Measurement of Epidermal Turnover in Humans

Kazuhisa Maeda

School of Bioscience and Biotechnology, Tokyo University of Technology, 1404-1 Katakuramachi, Hachioji City, Tokyo 192-0982, Japan; kmaeda@stf.teu.ac.jp; Tel.: +81-42-637-2442

Received: 24 September 2017; Accepted: 7 November 2017; Published: 10 November 2017

Abstract: This report describes a new and simple technique to detect alterations in the rate of turnover in the epidermis without using any toxic chemical, such as a radiolabeled material. The method involves measuring the time course of the decrease of darkening of an ultraviolet A-irradiated site, compared with a non-irradiated control site. The turnover time of the persistent pigmentation on the inner side of the male forearm was 36.2 ± 6.2 days (age: 37.3 ± 11.3 years, mean ± standard deviation, n = 6), which is in reasonable agreement with the epidermal turnover time previously measured by injecting [^{3}H] thymidine into human skin.

Keywords: epidermal turnover; turnover time

1. Introduction

Epidermal turnover is the process of the generation of keratinocytes in the basal layer of the epidermis and their eventual loss as corneocytes from the skin surface. The time taken for this process to be completed is called the epidermis turnover time or rate. This turnover plays an important role in maintenance of the skin's barrier function.

Melanin pigments, which are induced by ultraviolet (UV) exposure, are lost with the dead cells during epidermal turnover. If the turnover rate declines, they will remain apparent on the skin for longer. The rate of turnover is almost constant in healthy skin, and the stratum corneum transit time is approximately 20 days in young adults, although it gradually increases with age, lengthening by more than 10 days in older adults [1]. It is also known that the turnover time is accelerated in areas exposed to the external environment, such as the face, compared with non-exposed areas. Thus, measurement of the rate of turnover can provide an important clue to the health of the skin.

Various methods to measure the turnover rate have been proposed. For example, Weinstein et al. injected [^{3}H] thymidine into human skin, based on the fact that [^{3}H] thymidine is taken up by multiplying cells [2]. However, this method is unsuitable for routine use. Another approach was to measure the decline rate of the fluorescence intensity of dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride), which reacts with primary amino groups in both aliphatic and aromatic amines to produce stable blue- or blue-green fluorescent sulfonamide adducts of the stratum corneum [3,4]. It is reported that the turnover time of forearm stratum corneum using the dansyl chloride method is 13.9 ± 2.9 days (age: 30.0 ± 7.3 years, mean ± standard deviation, n = 14, correlation coefficient R = 0.3382) [5]. Dansyl chloride is known to be toxic, and so dihydroxyacetone has been used as a substitute [6]. However, these reagents specifically dye only the stratum corneum when they are applied onto the skin; thus, the turnover of stratum corneum can be measured, but not the turnover of the epidermis. Because of the safety problems and other disadvantages of these methods, there is still a need for a safe and simple method of measuring epidermal turnover. This paper presents a novel method based upon the measurement of the time course of the decrease in melanin pigmentation following exposure of the skin to UVA.

2. Materials and Methods

2.1. Subject

Healthy Japanese men with Fitzpatrick skin type III and IV, who had no history of photosensitivity and were taking no medication, participated in this study. The untanned inside of the forearm was used for the study. All volunteers had given their informed consent to participate, and the study protocol was approved by the Ethics Committee.

2.2. Light Source

The UVA source used in this study was a 300 W xenon solar UV solar simulator (Multiport 601, Solar Light Company, Philadelphia, PA, USA).

2.3. Measurements of Epidermal Turnover Time, Erythema, and the Amount of Water Transpiration from the Surface of Skin

The inner side of the forearm of six healthy Japanese (male, aged 26 to 51 years, 37.3 $\pm$ 11.3 (average age $\pm$ standard deviation)) was irradiated with 10, 20, 40 J/cm^2 of UVA. The decline in darkness of the skin was parallel to the UVA dose used. The irradiation with 40 J/cm^2 was sufficient to induce darkening without inflammation and apoptosis by the histochemical study, using a UV solar simulator [6]. This amount of irradiation was equal to the amount of UVA exposure during 2 to 3 h in the daytime in summer, as measured with a diffraction grating spectroradiometer (MS-701, EKO Instruments, Tokyo, Japan). The degree of skin darkening was measured by using a Mexameter MX16 (Courage and Khazaka Electronic, Köln, Germany) immediately before and after, and at 14, 21, 28, and 35 days after irradiation. The change in skin darkening was evaluated in terms of ΔM, which was calculated by subtracting the Melanin Index (M) immediately before irradiation from M (the value of the irradiation site $-$ the value of the perilesional site) at each measurement time. The epidermal turnover time is equal to the time required for ΔM to reach 0.

In order to confirm that inflammation did not occur, the Mexameter (Courage and Khazaka Electronics, Cologne, Germany) and a Tewameter TM-210 (Courage and Khazaka Electronics) were used to measure the skin erythema (Erythema Index (E)) and the water transpiration (trans-epidermal water loss (TEWL)) at the skin surface immediately before and after irradiation. The change in skin erythema, ΔE, was calculated by subtracting the value of E immediately before irradiation from that a day later. The change in the trans-epidermal water loss, ΔTEWL, was similarly calculated by subtracting TEWL immediately before irradiation from that a day later. The significance of differences between irradiated and non-irradiated sites was determined by use of the unpaired *t*-test. The protocol of this experiment was approved the institutional ethics committee, and informed consent was obtained from all volunteers.

2.4. Statistical Analysis

Paired (two-tailed) *t*-test was used to compare the means of the observed values.

3. Results

3.1. Measurement of Epidermal Turnover Time

The time course of ΔM for each individual and its mean value are shown in Figure 1. Blackish brown persistent pigmentation (PP) appeared on all six healthy Japanese individuals following irradiation. The initial dark gray immediate pigment darkening (IPD) disappeared within a few minutes. The time course of ΔM was consistent with our previous findings at lower energy, 16.8 J/cm^2 [6]. The degree of pigmentation gradually decreased, indicating that new melanin biosynthesis had little influence. The statistical differences were computed between the value obtained 14 days after irradiation and each value obtained at 21, 28, and 35 days after irradiation, because the

reported turnover time of the stratum corneum was 13.9 days [5]. All values were shown to have statistical significance. The epidermal turnover time obtained in this experiment was 36.2 ± 6.2 days (average ± standard deviation), which is consistent with values in the literature (39 days) measured by using a radiolabel [2]. Given that the correlation coefficient (R) between age and epidermal turnover time was 0.3247, age and epidermal turnover time can be said to be correlated well.

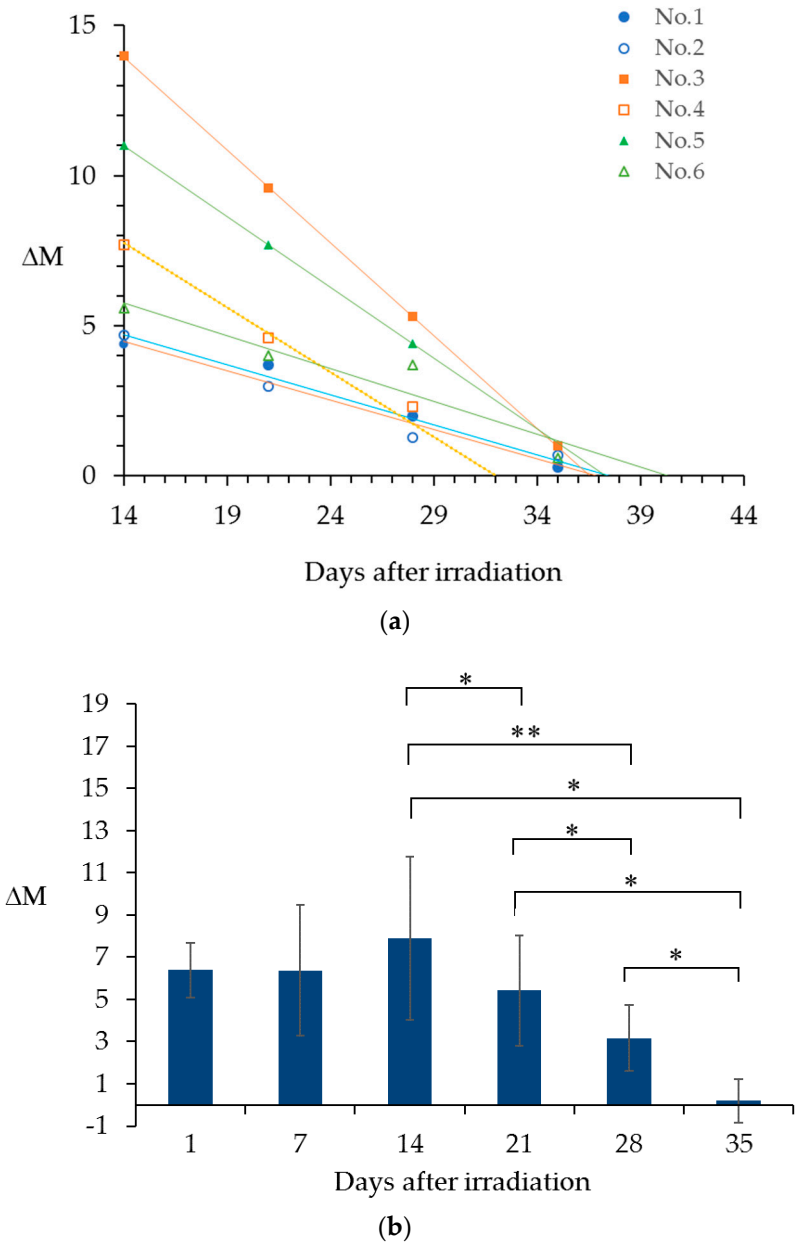

Figure 1. Time course of decrease in skin darkening produced by UVA irradiation of the inner male forearm. (**a**) The results are expressed as the ΔM of each subject. The change in skin darkening was evaluated in terms of ΔM, which was calculated by subtracting the Melanin Index (M) immediately before irradiation from M at each measurement time; (**b**) The results are expressed as the mean ± standard deviation of ΔM of six subjects. * $p < 0.05$ vs. 14 days after irradiation, and ** $p < 0.01$ vs. 14 days after irradiation.

3.2. Measurements of Erythema and Water Transpirationity

The changes in erythema and TEWL in individual volunteers are shown in Figures 2 and 3, respectively. There was no significant difference in erythema or TEWL between irradiated and non-irradiated sites ($p = 0.2126$ and $p = 0.7003$, respectively). Thus, it appears that the conditions used in this method do not cause any significant skin injury.

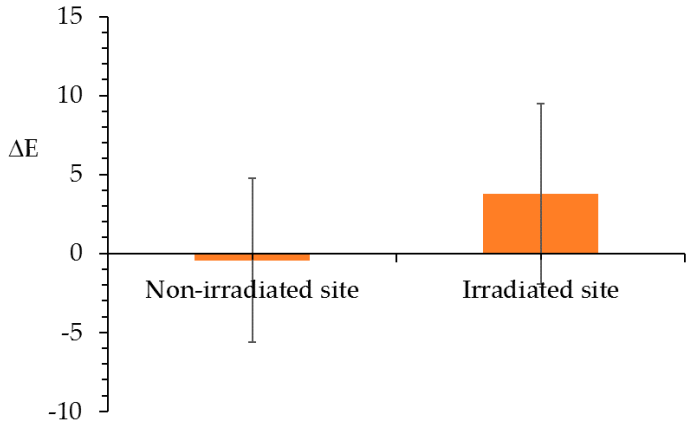

Figure 2. Changes in erythema at the UVA-irradiated site and non-irradiated site. The results are expressed ΔE, which was calculated by subtracting the value of Erythema Index (E) immediately before irradiation from that a day later, as the mean ± standard deviation of six subjects.

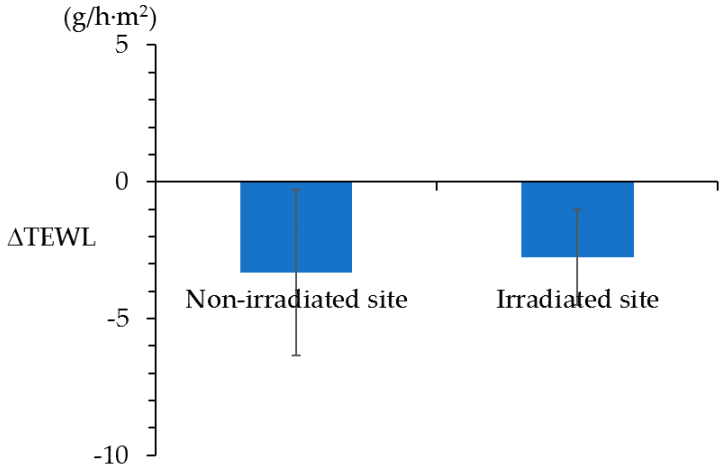

Figure 3. Changes in trans-epidermal water loss (TEWL) at the UVA-irradiated site and non-irradiated site. The results are expressed as ΔTEWL, which was calculated by subtracting the value of TEWL immediately before irradiation from that a day later, as the mean ± standard deviation of ΔTEWL of six subjects.

4. Discussion

Persistent dark-brown pigmentation appears soon after exposure to sunlight, and thereafter subsides slowly. This PP caused by UVA is considered to be associated with the photo-oxidation of melanin precursors in the basal layer of epidermis [7]. The dose of 40 J/cm^2 of UVA used here was sufficient to induce pigmentation without causing inflammation. Further, this dose of UVA is less than that required to cause melanocyte activation and stimulation of melanogenesis [8].

In this experiment, we estimated the epidermal turnover time from the time required for the disappearance of melanin induced by UVA exposure of the skin. We obtained a value of 36.2 ± 4.0 days (average ± standard deviation), which is similar to the literature value of 39 days (age: 40 years) [2]

or 45 days based on the model of desquamation rates of the stratum corneum and the consequent obligatory turnover time of the keratinocyte layer [9]. Therefore, our method appears to be reliable, and does not require the use of any toxic chemical. The erythema and TEWL data further indicate that skin injury is minimal, if any. Moreover, insolation experienced on a day-to-day basis in summer causes similar skin darkening in healthy persons, so the UVA dose can be considered safe. The method described here should be useful in evaluating cosmetics, medicated cosmetics, cosmeceuticals, and pharmaceuticals for cutaneous application. It should also be suitable for studies on morphological changes in the skin in dermatosis and hyperkeratosis, as well as for studies on skin aging.

We estimated the epidermal turnover time from the time required for the disappearance of melanin induced by UVA exposure of the skin. The epidermal turnover time obtained in this method was consistent with values in the literature measured by using a radiolabel. This method should be useful in detecting alterations in the rate of cell renewal in the epidermis without using any toxic chemical. This method needs to be improved in the measurement of the epidermal turnover on the face of female subjects, because most of women prefer not to have dark spots for up to two months on their face. In future studies, this measurement system will be generalized by increasing the number of subjects.

5. Conclusions

The turnover time of the PP on the inner side of the male forearm was 36.2 $\pm$ 6.2 days (age: 37.3 $\pm$ 11.3 years, mean $\pm$ standard deviation, n = 6), which is in reasonable agreement with the epidermal turnover time previously measured by injecting [^{3}H] thymidine into human skin.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Grove, G.L.; Kligman, A.M. Age-associated changes in human epidermal cell renewal. *J. Gerontol.* **1983**, *38*, 137–142. [CrossRef] [PubMed]
2. Weinstein, G.D.; McCullough, J.L.; Ross, P. Cell proliferation in normal epidermis. *J. Investig. Dermatol.* **1984**, *82*, 623–628. [CrossRef] [PubMed]
3. Johannesson, A.; Hammar, H. Measurement of the horny layer turnover after staining with dansyl chloride: Description of a new method. *Acta Derm. Venereol.* **1978**, *58*, 76–79. [PubMed]
4. Finlay, A.Y.; Marshall, R.J.; Marks, R. A fluorescence photographic photometric technique to assess stratum corneum turnover rate and barrier function in vivo. *Br. J. Dermatol.* **1982**, *107*, 35–41. [CrossRef] [PubMed]
5. Takahashi, M.; Machida, Y.; Marks, R. Measurement of turnover time of stratum corneum using dansyl chloride fluorescence. *J. Soc. Cosmet. Chem.* **1987**, *38*, 321–331.
6. Piérard, G.E.; Piérard-Franchimont, C. Dihydroxyacetone test as a substitute for the dansyl chloride test. *Dermatology* **1993**, *186*, 133–137. [CrossRef] [PubMed]
7. Maeda, K.; Hatao, M. Involvement of photooxidation of melanogenic precursors in prolonged pigmentation induced by ultraviolet A. *J. Investig. Dermatol.* **2004**, *122*, 503–509. [CrossRef] [PubMed]
8. Beitner, H. The effect of high dose long-wave ultraviolet radiation (UVA) on epidermal melanocytes in human skin: A transmission electron microscopic study. *Phodermatology* **1986**, *3*, 133–139.
9. Bergstresser, P.R.; Taylor, J.R. Epidermal 'turnover time'—A new examination. *Br. J. Dermatol.* **1977**, *96*, 503–509. [CrossRef] [PubMed]