Original Article

DOI: 10.5582/ddt.2012.v6.3.157

Evaluation of skin surface hydration state and barrier function of stratum corneum of dorsa of hands and heels treated with PROTECT X2 skin protective cream

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ABSTRACT: Skin roughness is a term commonly used in Japan to describe a poor skin condition related to a rough and dry skin surface that develops as a result of various damaging effects from the environment or skin inflammation. Recovery from skin roughness requires skin care for a long period, thus it is important to prevent development of such skin changes. PROTECT X2 contains agents used for a protective covering of the skin from frequent hand washing or use of alcohol-based disinfectants. These unique components are also thought to be effective to treat skin roughness of the dorsa of the hands and heels. In the present study, we evaluated the effectiveness of PROTECT X2 to increase skin surface hydration state, as well as enhance the barrier function of the stratum corneum of the dorsa of the hands and heels in elderly individuals. A total of 8 elderly subjects and their caretakers without any skin diseases participated in the study. They applied PROTECT X2 by themselves to the dorsum area of 1 hand and heel 3 to 5 times daily for 1 month, while the opposite sides were left untreated. We measured stratum corneum (SC) hydration and transepidermal water loss (TEWL) before beginning treatment, then 1 week and 1 month after the start of treatment to compare between the treated and untreated skin. SC hydration state after applications of PROTECT X2 was 1.5- to 3.0-fold higher than that of the untreated skin in the dorsa of both hands and heels, indicating that the moisturizing ingredients accompanied by water were replenished in those areas where the cream was applied. Also, TEWL in the dorsum of the hands was 17.0-27.9% lower on the treated side, indicating improvement in SC barrier function. On the basis of these findings, we concluded that PROTECT X2 enhances water-holding in the SC and aids the barrier function of the skin in the dorsum of the hands. In addition, we consider that this formulation is useful for not only protecting the hands from the effects of such agents as detergents and alcohol-based disinfectants, but also for protecting heel skin covered by a thick SC from dry and cold conditions such as those encountered in winter. However, since the SC in that area is much thicker than that of the hands, the barrier function was not significantly improved within 1 month of daily treatments.

Keywords: Stratum corneum, skin protective cream, transepidermal water loss, water content

1. Introduction

"Skin roughness" is a commonly utilized term in Japan for disturbed skin surface, which develops from synergistic interactions of various factors such as dryness and inflammation. The skin is composed from external to internal of the stratum corneum (SC), epidermis, dermis, and subcutaneous tissue. The SC covers the skin surface as an extremely thin membranous barrier and has an important protective role against the external environment (*1*). Approximately 30% of the content of the SC is water, which functions to maintain smoothness and softness of the skin surface even under dry external environmental conditions (*2*). Thus, the SC has an important barrier function to prevent the infiltration of harmful substances from outside of the body and also prevents water loss from the living tissues that it covers (*1*).

Various substances in the SC play a role to maintain hydration of the SC. They consist of sebum secreted from the sebaceous glands to cover the skin surface, except for the palms and soles, low-molecular weight substances termed natural moisturizing factor (NMF), which are chiefly composed of highly hygroscopic amino acids present in corneocytes (*3*), and intercellular lipids that spread between the corneocytes, such as ceramides,

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cholesterol, and fatty acids, which inhibit water loss from the tissues and prevent NMF effusion to the outside of the cells (*4*). The SC is produced by proliferation and differentiation of keratinocytes composing the epidermis as a result of epidermal turnover, which normally occurs over a period of 28 days in most parts of the body, such as the trunk and limbs. However, once inflammation involves the epidermis, the cell cycle shortens to induce increased keratinocyte proliferation, which causes reductions in the amounts of intercellular lipids and NMF, leading to a decrease in water content in the SC. Under such conditions, so-called dry skin develops, in which the skin surface texture is disturbed to induce exfoliation of the corneocytes as a mass, namely scaling. Thus, it is inevitable that a decrease in SC barrier function protecting the body from desiccation occurs due to a dry external environment (*5*).

For objective and non-invasive evaluations of skin surface conditions, measurements of high-frequency conductance and transepidermal water loss (TEWL) are widely used (*6-8*). With the former, it is possible to determine the hydration state of the skin surface, *i.e.*, water content in the superficial portion of the SC that faces the environment. Thus, it can also be used to determine the efficacy of a cosmetic base on water content in the SC when measured under constant temperature and moisture conditions (*6,7*). In contrast, TEWL measurement assesses the barrier function of the SC by determining the amount of water that evaporates from the skin surface and is lost from water-saturated skin tissues (*8*). It has been reported that such measurements should be made without the influence of perspiration or airflow, at around 20°C (*9*). Since the water content of the superficial portion and barrier function constitute the vital functional characteristics of the SC that covers the skin surface, these measurements are generally performed in combination. Recently, for evaluating skin surface morphology, or skin texture, imaging analysis using replicas has also been utilized (*10*).

When skin roughness is left untreated, an increase in the risk of inflammation or infection can develop due to the formation of cracks in the SC. Thus, it is considered that the fundamental issue for maintaining healthy skin is moisturizing to keep the skin surface smooth and soft, and prevent development of cracking. Cosmetic products produced for improving skin roughness are generally composed of water, oil, and moisturizing agents, which are suitable for maintaining the balance of water, NMF, and lipids on the skin surface. These are broadly interpreted as preparations that maintain homeostasis of the skin, thus cosmetic products and quasi-drugs used for maintaining, restoring, and improving the function of epidermal tissue have been actively developed. An agent that improves rough skin is sometimes used alone, but mostly in combination with multiple moisturizing agents such as highly concentrated glycerin and propylene glycol, and anti-inflammatory agents such as glycyrrhizinate and lysozyme chloride for suppressing minor inflammation.

In elderly individuals, a relatively thick SC is formed in the skin surface as epidermal proliferation reduces and differentiation slows with poor production of the NMF in the epidermis with aging, resulting in accumulation of dry corneocytes in the superficial portion of the SC. Accordingly, corneocytes with low NMF content tend to remain on the skin surface of elderly subjects due to reduced activity of the proteolytic enzymes to facilitate to reduce their adhesion to each other (*11*). Moreover, sebum secretion begins to decline in the fifth decade of life, and skin changes termed senile xerosis begin to develop in the dry and cold conditions of winter. In most affected individuals, the skin surface of the back, hips, and legs becomes dry and cracked, inducing itchiness (*11*). Furthermore, in bedridden or wheelchair-bound elderly individuals, the incidence of pressure sores, so-called bedsores, also increases. Pressure sores is a condition in which blood flow in the skin deteriorates due to compression for long periods of time while sitting in a chair or lying on a bed, causing cracking of the SC, which then spreads from the skin surface to subcutaneous tissue and maintained in the presence of poor blood circulation. Moreover, when the skin is stimulated by friction, cracking of the thickened SC may occur, facilitating the development of pressure sores. In the case of a heel affected by pressure sores, walking becomes difficult, affecting bodily movement. Since recovery from skin injury requires a long period of time in elderly individuals, it is important to prevent the development of these skin conditions.

PROTECT X2 contains high concentrations of glycerin and dipropylene glycol, generally employed as moisturizing components, as well as glycereth-25 PCA isostearate, stearyl glycyrrhetinate, and tocophenyl acetate, which are widely used as anti-inflammatory components. In addition, it is worth noting that PROTECT X2 contains 3 other components, aminoethyl aminopropylmethylsiloxane-dimethylsiloxane copolymer from a silicon series, polyvinyl pyrrolidone from a vinyl series, and perfluoropolyether from a fluorine series, which are used to produce a protective covering for skin from external damaging factors such as hand washing and use of alcohol-based disinfectants.

In the present study, we examined the efficacy of this formulation for forming a protective skin covering, especially to enhance SC barrier function on the dorsum of the hand. We examined the effectiveness of PROTECT X2 for water holding when applied to the dorsum of the hands of elderly individuals. In addition, we examined its effectiveness on heel skin, the plantar area where the skin is covered by a uniquely thick SC, which requires sufficient hydration to remain soft and flexible, and resists strong external forces, for avoiding formation of cracking or deep fissures, which particularly occur in elderly individuals who tend to have thicker SC than young healthy individuals (*11*).

2. Materials and Methods

2.1. *Test agent and measuring instruments*

PROTECT X2 (Newhair Cosmetic Material Co., Ltd., Tokyo, Japan), a quasi-drug, was the agent examined in this study. It is mainly composed of glycerin, dipropylene glycol, glycereth-25 PCA isostearate, stearyl glycyrrhetinate, tocophenyl acetate, aminoethylaminopropylmethylsiloxane-dimethylsiloxane copolymer, polyvinyl pyrrolidone, and perfluoropolyether. To measure the hydration state of the skin surface, we used a SKICON-200EX (I.B.S Co., Ltd., Shizuoka, Japan). For determination of TEWL, a Tewameter® TM300 (Courage + Khazaka Electronics GmbH, Cologne, Germany) was employed. These instruments were operated in accordance with the enclosed instruction manuals and the instrumental measurements were made in an environment controlled to a constant temperature and humidity (21-23°C, 43-50% relative humidity).

2.2. *Subjects*

A total of 8 elderly individuals and their caretakers (1 male, 7 females) ranging from 29-85 years old (mean 52.8 years) participated in the study as the subjects. None had any special skin diseases, but demonstrated mild to moderate skin roughness. Informed consent was obtained after explaining verbally and in writing the purpose and methods of the experiment, as well as handling of the data obtained from the experimental results. The present study was approved by the Ethical Committee of Chiba Institute of Science (Approval No. 23-2).

2.3. *Method of application of PROTECT X2*

The study was performed in the period from December 2011 to January 2012. The subjects applied PROTECT X2 3-5 times daily to the dorsa of one of their hands and one of their heels for a period of 1 month, with the application amount set at 0.6 mL per dose. As a control, nothing was applied to the contralateral hand and heel throughout the 1-month study period. SC hydration state and TEWL in the SC were determined 5 times at each measuring site, from which the mean value was calculated after excluding the maximum and minimum values. The measurement obtained immediately before the start of the application was considered to be the initial value. Similar measurements were conducted again 1 week and 1 month after the start of the applications. The measurements were made in principle when the subjects were calm, in an environment controlled at constant temperature and humidity (21-23°C, 43-50% relative humidity). The maximum and minimum temperature and humidity at each measuring point were also recorded.

2.4. *Statistical analysis*

For testing the significance of the mean values between the groups, Student's paired *t*-test was used, while Pearson's correlation coefficient was used for analyzing correlations. For correlation analysis of the mean values with temperature and humidity, Pearson's correlation coefficient was used. The level of significance in Student's *t*-test was 5%. For testing the correlation coefficient (*r*), Fisher's *r* to *z* transformation was used. Microsoft® Office Excel 2007 (Microsoft Japan Co., Ltd., Tokyo, Japan) and KaleidaGraph® 3.6 (Hulinks Inc., Tokyo, Japan) were used, as appropriate, for statistical calculations.

3. Results

3.1. *Changes in skin surface hydration state with PROTECT X2 applications*

Before the start of the PROTECT X2 applications, there was no significant difference between the dorsum of the pre-treated and the untreated hands (96.8 ± 18.9 *vs.* 85.8 ± 20.2 μS; mean ± standard error), or between that of the pre-treated and the untreated heels (12.7 ± 3.9 *vs.* 8.4 ± 2.2 μS). After 1 week of PROTECT X2 applications, the skin surface hydration state of the dorsum of the hands became 169.9 ± 54.8 μS, which was 2.6-fold greater than that of the untreated side (66.5 ± 25.1 μS) ($p < 0.05$). Furthermore, after 1 month of daily applications, the hydration state of the treated side was 68.0 ± 14.1 μS, which was 1.5-fold greater than that of the untreated side (45.3 ± 12.3 μS) ($p < 0.01$) (Figure 1A). Meanwhile, in the 7 subjects who applied PROTECT X2 to one of their heels daily for 1 week, the hydration state in the treated side was 16.7 ± 3.2 μS, which was 3.0-fold greater than that of the untreated side (5.5 ± 1.2 μS) ($p < 0.01$). One month after the start of the applications, the hydration state in the treated heel was 20.4 ± 4.2 μS, or 2.8-fold greater than that of the untreated side (7.2 ± 1.3 μS) ($p < 0.05$) (Figure

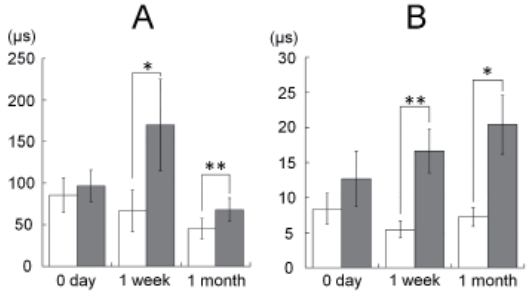

Figure 1. Changes in water content in corneocyte layer with PROTECT X2 application. A) Water content in dorsum of hand ($n = 8$). **B)** Water content in heel ($n = 7$). Open column, untreated side; closed column, treated with PROTECT X2. Error bar, mean ± standard error. * $p < 0.05$, ** $p < 0.01$.

1B). These findings suggest that PROTECT X2 was effective to increase the SC hydration state in the dorsa of the hands and heels.

3.2. *Changes in TEWL with PROTECT X2 applications*

Before the start of the PROTECT X2 applications, there was no significant difference between the dorsum of the pre-treated and the untreated hands (13.4 ± 1.8 *vs.* 14.3 ± 1.7 g/hm^2), or between that of the pre-treated and the untreated heels (30.6 ± 3.9 *vs.* 34.2 ± 5.4 g/hm^2). We examined TEWL in the dorsum of the hands of 8 subjects. After 1 week of daily applications of PROTECT X2, that value was 13.7 ± 1.6 g/hm^2 in the treated side, which was 27.9% lower than that of the untreated side (19.0 ± 3.3 g/hm^2) ($p < 0.05$). Furthermore, TEWL at 1 month after starting PROTECT X2 applications was 15.1 ± 2.4 g/hm^2 in the treated side, 17.0% lower than that in the untreated side (18.2 ± 2.2 g/hm^2) ($p < 0.05$) (Figure 2A). In contrast, in the 7 subjects who applied PROTECT X2 to their heel, TEWL after 1 week was 35.4 ± 2.5 g/hm^2, which was 17.2% higher than that in the untreated side (30.2 ± 1.9 g/hm^2). TEWL after 1 month of daily PROTECT X2 applications was 36.0 ± 5.0 g/hm^2 in the treated heel, 15.0% higher than that in the untreated side (31.3 ± 4.0 g/hm^2) (Figure 2B). On the basis of these findings, we concluded that PROTECT X2 helped to decrease TEWL in the dorsum of the hand, whereas it increased TEWL in the heel skin in the present subjects.

4. Discussion

So-called healthy skin indicates a condition in which the skin surface is firmly and smoothly covered with SC, and an adequate amount of moisture is retained. Measurements of water content in the superficial portion of the SC as well as TEWL can be employed to objectively evaluate the condition of the skin surface. The former is used as an index for moisture-holding capacity, while the latter represents the barrier function of skin (*6-8*). Accordingly, these measurements together are suitable for determining such conditions such as xerosis, in which moisture in the skin is lost during the dry period in winter, and exfoliation of the superficial portion of the SC occurs due to repeated movements of the skin that produce friction as well as cracking in the skin surface.

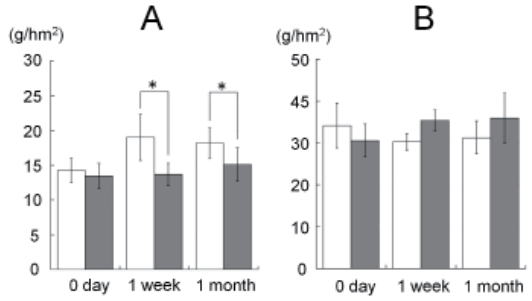

Figure 2. Changes in TEWL in corneocyte layer with PROTECT X2 application. A) TEWL in dorsum of hand ($n = 8$). **B)** TEWL in heel ($n = 7$). Open column, untreated side; closed column, treated with PROTECT X2. Error bar, mean ± standard error. * $p < 0.05$.

In the present subjects, the level of hydration of skin surfaces repeatedly applied with PROTECT X2 was frequently found to range from 1.5- to 3-fold greater in conductance measurements as compared to the untreated sides for both the hands and heels. The mean hydration state at 1 week after the start of applications was the highest at 169.9 ± 54.8 μS, though the early values also showed the largest standard error ranges. Thus, we think that the extent of increase in SC hydration varies between individuals due to the fact that only 1 week had passed after the start of application. Although the water content in the SC of the heel was highest after 1 month of treatment (mean 20.4 μS), it was only about one-tenth of the maximum value (169.9 μS) measured in the dorsum of the hands. This corresponded to the fact that the SC of the heel is distinct from that of the other portions of the body except for the palms, because, like palmar skin, it is several times thicker than that of the dorsum of the hands an other areas of the body. Over time, hydration state in the heel tended to increase gradually from 16.7 μS at 1 week to a peak value at 1 month after the start of the applications (Figure 1B) (*12*).

In our present investigation of the correlation between PROTECT X2 treated and untreated sites, strong correlations were confirmed after 1 week (0.89) and 1 month (0.96) (both, $p < 0.01$) in the dorsum of the hands, which were statistically significant. These findings indicate that higher levels before starting treatment resulted in higher levels after the applications. Thus, we think that the higher hydration state in the treated side was due to the daily applications of PROTECT X2 to the dorsum of the hands. Meanwhile, no such correlation was observed at any time points for the heels. Thus, it is important to consider the unique water distribution in the palmo-plantar SC, as a thick low hydrated portion has been found in the upper and middle part of the SC in that area (*12*). In addition, it is possible that perspiration due to physical and mental changes in the subjects (*9*), individual differences in the timing of putting on footwear such as socks after applications, or differences in moisture retention because of different footwear materials might have also affected our results. We found a large difference regarding hydration state in the SC between the treated and untreated sides at 1 week after the start of the applications in both hands and heels. However, our findings indicate that daily PROTECT X2 applications for 1 week are effective to enhance the moisture retention capacity of the SC. We already studied that the

moisture retention of the SC after its single application are continuous with 3 h at least (data not shown).

TEWL values for the dorsum of the hands applied with PROTECT X2 were 17.0-27.9% lower than those of the untreated hands, whereas TEWL values for the treated heels were 15.0-17.2% higher than those of the untreated heels. The mean TEWL of the treated side remained nearly constant throughout the experimental period (13.7 g/hm^2 at 1 week, 15.1 g/hm^2 at 1 month), whereas that of the untreated side showed an increasing tendency, suggesting a worsening of the barrier function when it remained untreated. We concluded that this was because the protective covering provided by PROTECT X2 on the SC prevented water loss from the dorsum of the hands, indicating that repeated applications of PROTECT X2 prevent weakening of the SC barrier function (Figure 2A). This has also been reported when measurements were done with dry skin (*13,14*). In contrast, TEWL in the treated heels remained higher than that in the untreated side throughout the experimental period. We speculated that this reflected the supply of water and moisturizing ingredients by PROTECT X2 to the SC of the heel. Because the SC of the heel is much thicker than that in the dorsum of the hands, it is likely that the SC cannot be replaced totally by new corneocyte layers within 1 month (*15*). Accordingly, we think that the barrier function of plantar skin might not be improved within the treatment period, as intercellular lipids such as ceramide did not become normalized within the 1-month experimental period. Thus, it may require more time to improve the barrier function in the heel (Figure 2B).

We also investigated the correlation between the treated and untreated sides over time. For the dorsum of the hands, the correlation was 0.86 ($p < 0.01$) at 1 week after the start of application and 0.88 ($p < 0.01$) at 1 month, which were statistically significant, suggesting that a smaller correlation before treatment resulted in a smaller correlation after the applications. We concluded that the lower TEWL value in the treated side of the hands was a result of barrier function improvement induced by PROTECT X2 applications. However, no such correlations were found for TEWL in the heel. It is possible that a total lack of sebum excretion and low amounts of intercellular lipids in addition to the unique sweat glands that have relatively abundant secretion in the heel areas might have affected these measurements (*9*), in addition to the thickness of the heel SC, as noted above.

Neither water content nor TEWL had any correlation with room temperature or humidity (water content: $p = 0.30$-0.96, TEWL: $p = 0.15$-0.88). Accordingly, we think that our study was performed under proper environmental conditions and obtained highly reliable results. In addition, visual assessments throughout the measurement period confirmed that skin in the untreated sides had greater amounts of scaling and chapped skin, as well as bleeding from cracks over time (Figure 3). Meanwhile, hands and heels applied with PROTECT X2 maintained adequate moisture levels. Skin tenderness is closely related to water content in the SC (*2*), thus it is useful to measure skin surface hydration for evaluating skin roughness.

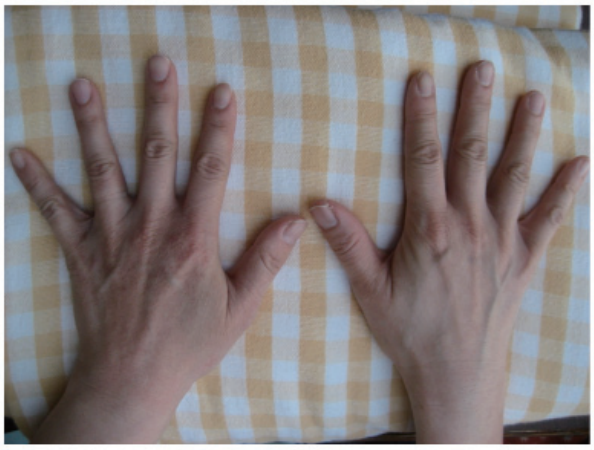

Figure 3. Visual observations throughout the measurement period. The untreated hands had greater amounts of scaling and chapped skin, as well as bleeding from cracks after 1 month (the left hand). Meanwhile, hands applied with PROTECT X2 maintained adequate moisture levels.

Based on the present findings, we concluded that daily applications of PROTECT X2 increases SC hydration state in the skin of patients with senile xerosis and improves the barrier function in the dorsum of the hand. Thus, it is reasonable to consider that this preparation is effective for not only protecting the hands from detergent and use of alcohol-based disinfects, but that it can also protect heels from winter dryness and friction.

Acknowledgements

I wish to thank Nozomi Suzuki (Bachelor of Pharmacology) and Mayumi Shibayama (Bachelor of Pharmacology) for their assistance with the measurements. I also gratefully acknowledge Dr. Hachiro Tagami, Professor Emeritus of Tohoku University, for the cooperation and guidance in designing the investigation and completing the study.

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(Received May 31, 2012; Revised June 16, 2012; Accepted June 18, 2012)