Detection of albumin using skin blotting as a measure of skin barrier function

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Abstract

Measurement of transepidermal water loss (TEWL) is the gold standard for the evaluation of skin barrier function. However, it is difficult to obtain stable values in the clinical setting. In this study, we evaluated the use of albumin detection by skin blotting as a new measurement method for determination of skin barrier function. We analyzed the correlation between TEWL values and detected the intensity of albumin staining by skin blotting in animal models and humans. The albumin intensities were significantly correlated with TEWL values in samples of back skin from mice \( (r = 0.56, p = 0.02) \). In addition, the albumin intensities were also correlated with TEWL values in human skin \( (r = 0.73, p < 0.01) \). Thus, albumin intensity using skin blotting was a noninvasive and stable method for evaluating skin barrier function as an alternative to TEWL.

Key words: albumin, animal experiment, skin assessment, transepidermal water loss

Introduction

Skin consists of the epidermis, basal membrane, dermis, and subcutis. Skin is composed of many types of cells and plays an important role in essential functions critical for health or wellbeing\(^1\), including skin barrier function, sensation, thermoregulation, immunological surveillance, biochemical reactions, and appearance. Skin barrier function is one of the most important roles of skin, serving to protect the body from external irritation or allergens and to inhibit the leakage of water and electrolytes from the body. In particular, the stratum corneum functions as the primary physical barrier and facilitates water retention\(^2\).

Recent studies have shown that tight junctions may also play a crucial role in skin barrier function. Tight junctions, a type of cell–cell junctions, are known to occur between simple epithelial cells and endothelial cells, thereby sealing neighboring cells and extracellular water in the stratum corneum, preventing irritation, and maintaining the balance of electrolytes or water\(^3\). The process regulating water loss in the skin is critical in humans. Notably, patients with atopic dermatitis show higher transepidermal water loss (TEWL) than healthy individuals\(^4\). Therefore, TEWL is one of the main indicators of skin barrier function.

Measurement of TEWL is the gold standard for evaluation of skin barrier function. Its repeatability is high in the experimental environment; however, it is difficult to obtain stable values in the clinical setting, in which the environment or patient condition may alter the results. Therefore, it is necessary to develop a new measurement method for evaluating the skin barrier function stably on the skin in the clinical setting. The skin blotting method, which quantifies the amount of protein leaking from the inside of the skin\(^5\), can noninvasively extract soluble molecules leaking from inside the skin barrier by attachment of a nitrocellulose membrane on the surface of the skin.
under wet conditions. The skin blotting method has been used in some studies to evaluate the inflammation of aged skin\(^6\)^7. Moreover, detection of the intensity of albumin, the major component of intercellular fluid, may reflect skin barrier function in humans. A previous study reported that albumin levels in the skin are well correlated with TEWL, as determined using tape stripping. However, in individuals with fragile skin, such as elderly individuals or infants, skin may be damaged by tape stripping.

Accordingly, in this study, we aimed to develop a new evaluation method of skin barrier function based on detection of albumin using the skin blotting technique.

**Methods**

1. Skin blotting in an animal model

To evaluate the correlation between albumin intensity using the skin blotting technique and TEWL values, skin blotting and TEWL measurements were carried out on the back skin of mice. Four 8-week-old male C57BL/6J mice (SLC Japan, Shizuoka, Japan) were maintained under controlled light (12-h light and 12-h dark) and temperature (25±2℃) conditions. Mice were allowed free access to food and water. Our animal experimental protocols were approved by the Animal Research Committee of the University of Tokyo. All animals were treated according to guidelines established by the Japanese Association for Laboratory Animal Science (1987).

The back skin of mice was shaved using electrical clippers. After 3 days, tape stripping, i.e., attachment and removal of adhesive plastic tape, was repeated 20 times on the back skin of mice. After every five repetitions of the tape stripping procedure, TEWL values were measured and skin blotting sampling was carried out repeatedly on the marked skin surface (Figure 1). A VapoMeter (VapoMeter SWL-4001 TS; Delfin Technologies, Kuopio, Finland) was used to measure TEWL values. TEWL values were measured repeatedly, and the mean value of three measurements was used for data analysis. For skin blotting, square (1×1 cm) pieces of nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) were used. Nitrocellulose membranes were prewetted with a drop of saline and were attached the back skins of mice with plastic tape for 10 min. Membranes were then collected and stored at 4℃ until analyses. In order to dry the wetted skin due to skin blotting, 15-min interval was required for the next procedure.

Immunostaining of nitrocellulose membranes for albumin was carried out using a SNAPi.d.2.0 protein detection system (Merck Millipore, Billerica, MA, USA). Nitrocellulose membranes were blocked with blocking solution (Blocking One; Nacalai Tesque, Kyoto, Japan) for 10 min and then reacted with anti-albumin antibodies conjugated with alkaline phosphatase (A114AN, American Qualex, San Clemente, CA, USA; dilution 1:50) for 10 min. Immunoreactivity was visualized by reaction with chemiluminescent AP substrate (Bio FX Laboratories, Owings Mills, MD, USA). The captured image with LumiCube (Liponics, Tokyo, Japan) was separated into RGB channels, and the average intensity of the signal was measured in the blue channel image by ImageJ 1.50i (National Institutes of Health, Bethesda, MD, USA).

2. Skin blotting with human skin

To evaluate the correlations between albumin intensities using the skin blotting technique and TEWL values, skin blotting and TEWL measurements were performed using human skin. We recruited adult volunteers from the University of Tokyo. Participants with skin disorders were excluded. Data for sex and age, were collected. This study was approved by the ethics committee of the University of Tokyo. Written informed consent was obtained from all participants.

The researcher marked a 2 cm × 2 cm square using a pen on the inside and outside of the forearm. We measured TEWL values and skin blotting within each square. TEWL was measured repeatedly, and the mean value of three measurements was used for data analysis. Subsequently, nitrocellulose membranes prewetted with a drop of saline were attached...
Membranes were then collected and stored at 4°C until analyses. Immunostaining was performed as described for the animal experiment.

**Statistical analysis**

The relationships between TEWL values and average intensities of albumin by skin blotting were analyzed in both the human and animal models. We performed a simple linear regression analysis using Excel 2013 (Microsoft Corporation). Results with \( P \) values of less than 0.05 were considered significant.

**Results**

1. **Demographic data for the human volunteers**

Nine participants (four males and five females) were recruited in this study. The median (range) age was 28 years (26–43 years).

2. **Relationship between TEWL values and albumin staining by skin blotting**

Figure 2 shows the results of albumin staining in mice and participants. The correlations between albumin intensity and TEWL values in animal dorsal skin and human forearm skin are shown in Figure 3 and 4, respectively. Albumin intensity was significantly correlated with TEWL values in both the back skins of mice \( (r=0.56, \ p<0.02) \) and forearm skin of humans \( (r=0.73, \ p<0.01) \).
between TEWL values and albumin staining of skin in mice and in human participants using the skin blotting in order to indicate feasibility of a non-invasive, simple skin blotting using albumin.

Yamane et al. reported that the amount of albumin collected from atopic skin by tape stripping was higher than that from normal skin and was correlated with both the local severity scores of atopic skin and TEWL. These findings revealed that the increased accumulation of albumin on the horny layer as well as the high TEWL values reflected the impairment of skin barrier function. Albumin is known to be present in the normal epidermis and is found throughout all nucleated epidermal layers. However, their results showed that albumin could not escape from the epidermis in healthy skin. Therefore, it was difficult to detect albumin in normal skin without tape stripping. However, the skin blotting method allows noninvasive collection of proteins within healthy skin that penetrate via the transepidermal route. Thus, targeting of albumin made it possible to evaluate skin barrier function in healthy individuals.

In this study, we selected albumin in the skin as a marker of skin barrier function because albumin is one of main components in the extracellular fluid and because albumin concentrations remain stable. We found that albumin intensity reflected skin barrier function, which controls epidermal permeability, due to stabilization of albumin concentrations. Therefore, albumin detection using skin blotting was shown to be an appropriate method for evaluating skin barrier function and may be suitable for use in the clinical setting because skin blotting is a noninvasive, stable, simple method.

This study had several limitations. We excluded participants with skin disorders. It was necessary to demonstrate that the levels of albumin reflected skin barrier function by skin blotting; further studies are needed to evaluate whether there are changes albumin levels in skin lesions. In addition, serum albumin has been attributed to inflammation, poor nutrition, and peritoneal protein loss. Hence, the albumin intensity measured by skin blotting may reflect the effects of both skin barrier function and illness severity. In future studies, we will examine feasibility of albumin detection using skin blotting more appropriate markers for evaluating skin barrier function in patients with various disorders.

Conclusions

In summary, we found that albumin detection on the skin of participants using the skin blotting was correlated with TEWL values. Thus, skin blotting may have applications in the evaluation of skin barrier function.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

原著

皮膚バリア機能測定としてのスキンブロッティング法によるアルブミン検出

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要旨

皮膚バリア機能の代表的な評価方法に経皮水分蒸散量（TEWL）があげられる。しかし、臨床の場において安定したTEWLの値を得ることはむずかしい。本研究では、皮膚バリア機能の新たな評価方法として、スキンブロッティングによるアルブミン検出法を評価するために、動物と人におけるTEWLとスキンブロッティングによるアルブミン検出強度の相関を分析した。結果として、マウスの背部皮膚ならびに人の前腕皮膚のアルブミン検出強度はTEWLと有意に相関していた（マウス；r=0.56, p=0.02, 人；r=0.73, p<0.01）。以上より、スキンブロッティングによるアルブミン検出強度はTEWLの代替としての皮膚バリア機能評価として非侵襲、安定的な方法といえる。

キーワード：アルブミン、スキンブロッティング、経皮水分蒸散量、動物実験