An Innovative Trio Strategy for Maintaining Skin Hydration Homeostasis

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Abstract

Various environmental and population diversity contribute to the complexities of dry skin conditions. Each skin layer requires a tailored hydration strategy in order to maintain the specific water balance. The purpose of this study is to investigate a three-in-one strategy of the stratum corneum, epidermis and dermis to maintain skin hydration homeostasis. A night blooming flower extract was used in this work as a new natural skin moisturizer. The coordinated increase in epidermal BLMH biosynthesis (NHEKs), intracellular taurine accumulation under hypertonic condition (NHEKs) and dermal HA production (NHDFs) was observed to address multi-layered hydration. Interestingly, in addition to maintaining skin water balance, these factors both have multiple biological activities, especially anti-inflammatory. In the meanwhile, their expression and bioactivities appear to be inversely correlated with age. These seem to confirm the important link between skin moisture loss, immune barrier function and aging. The trio strategy may represent a novel approach for maintaining skin hydration homeostasis.

Keywords: Skin hydration; Osmoregulation; Bleomycin hydrolase; Taurine; Hyaluronic acid

Introduction

Skin hydration is one of the basic requirements in the field of skin care. Maintenance of skin water balance is essential for cutaneous cells and barrier functions. Various environmental and physiological factors may contribute to the heterogeneity of skin dryness.

Several factors are believed to be involved in maintaining optimal water levels in skin, and the hydration strategies for different skin layers also need to be specific.

As the outermost layer of the epidermis, the hydration state of stratum corneum (SC) mainly depends on the content of intercellular lipids and natural moisturizing factors (NMF) in corneocytes [1]. Bleomycin hydrolase (BLMH) has been identified as critical for the degradation of filaggrin fragments into free amino acids [2], which are the major components (~40%) of NMF [3] in the upper SC. These NMF can absorb atmospheric moisture and retain water in the outermost layer of the epidermis [4, 5, 6].

Recent evidence has demonstrated that osmoregulation is closely related to cutaneous cell water balance. Epidermal keratinocytes are frequently investigated as potential osmoconformers, as these cells are subjected to extreme osmotic stress to properly regulate their cell volume and thus skin hydration [7]. Accumulation of the organic osmolyte taurine in keratinocytes is required to maintain cellular water balance under dry skin conditions [8]. Hyaluronic acid (HA), also known as nature's moisturizer, is one of the most hydrophilic molecules in nature [9]. Exogenous HA is widely used in the field of epidermal moisturizing, while endogenous HA plays a central role in deep skin hydration and elasticity [10].

Our previous studies have shown that crosstalk between cutaneous cells from different skin layers is critical for maintaining overall skin homeostasis. Therefore, a multi-layered hydration strategy seems to be particularly important to deal with the skin of different dehydration states under the exposome. The purpose of this study is to investigate a three-in-one strategy of the stratum corneum, epidermis and dermis to maintain skin hydration homeostasis.

Materials and Methods

Preparation of the natural skin moisturizer

A night blooming flower traditionally used in homeopathy has been identified. A new natural skin moisturizer (NSM) derived from an optimized water extraction of these air-dried flowers picked the morning after blooming. A LC-MS/MS based molecular networking of this natural skin moisturizer has been investigated to confirm that it is a concentrated source of small hydrated carbohydrates. The *in vitro* tests are conducted with the pure active matter of the novel natural skin moisturizer at 0.01%, 0.03% or 0.1%.

Epidermal keratinocyte culture and quantification of target proteins

Normal Human Epidermal Keratinocytes (NHEKs) obtained from abdominal skin were cultured in Keratinocytes Growth Medium 2 Kit (C-20111, PROMOCELL). The cultures were maintained in an incubator at 37°C, 5% CO_2 with an atmosphere saturated in humidity. NHEKs have been cultivated in monolayer until reaching confluency. Cells were pre-incubated during 48 hours in absence (control) or in presence of the new NSM. At the end of the pre-incubation period, BLMH protein was quantified in cell lysates using Bradford method. For the measurement of intracellular taurine level, Cells were pre-incubated during 72 hours in absence (control) or in presence of the new NSM. At the end of the pre-incubation period, the incubation medium is removed and replaced by hyperosomotic assay medium (KGM 400mOsM + Ca^{2+} 1.1mM + taurine 150µM). 72 hours after medium removal, cell monolayers are washed with cold hyperosmotic PBS. After rinsing, Intracellular taurine levels are measured in cell lysates using Taurine ELISA Kit.

Dermal fibroblast culture and quantification of HA

Normal Human Dermal Fibroblasts (NHDFs) obtained from abdominal skin were cultured in DMEM Medium (GIBCO) supplemented with 10% fetal calf serum (FCS) and antibiotics. The cultures were maintained in an incubator at 37°C, 5% CO_2 with an atmosphere saturated in humidity. NHDFs have been cultivated in monolayer until reaching confluency. Cells were pre-incubated during 48 hours in absence (control) or in presence of the new NSM. At the end of the pre-incubation period, HA was quantified in cell lysates using a sandwich ELISA assay (Cisbio Bioassays HYAL-ELISA).

Results

Stimulation of bleomycin hydrolase production in NHEKs

As mentioned before, the NMF content affects the hydration state of SC. In this study, we first evaluated the skin barrier hydration by measuring BLMH production in NHEKs. Under the experimental conditions, the new NSM at 0.01% and 0.03% significantly ($p<0.001$) increased intracellular level of BLMH in NHEKS in a dose-dependent manner (*Fig.-1*).

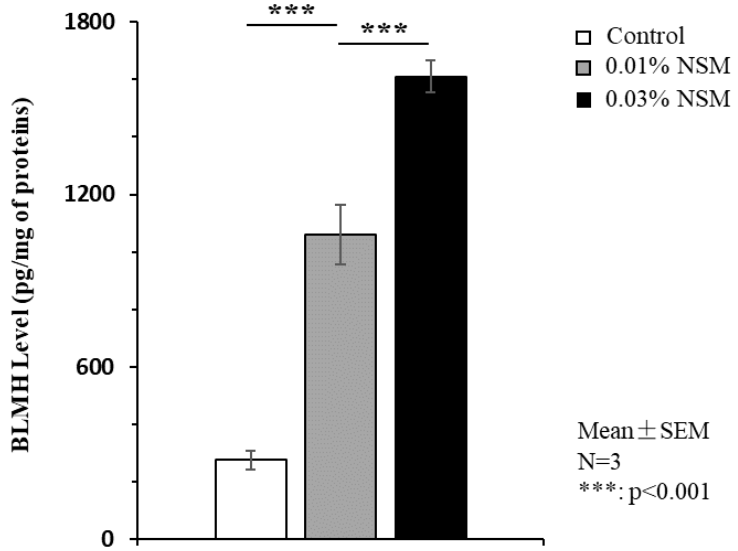

Fig.-1 Effect of the new NSM on BLMH production in NHEKs.
Results are expressed as pg of bleomycin hydrolase per mg of proteins. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.

Intracellular accumulation of taurine in NHEKs

To evaluated cellular water balance under dry skin conditions, NHEKs have been placed in a hypertonic medium to mimic the osmoregulatory processes that occur at cellular level when the skin is in a dry environment. Under our experimental conditions, the novel NSM at 0.01% and 0.03% significantly (*p<0.05 and p<0.01 respectively*) increased intracellular level of taurine in NHEKs in a dose-dependent manner (*Fig.-2*).

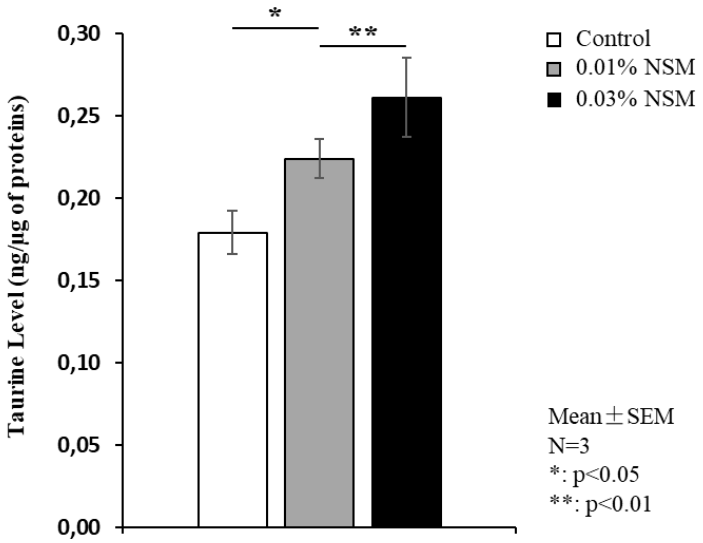

Fig.-2 Effect of the new NSM on intracellular accumulation of taurine in NHEKs.
Results are expressed as ng of taurine per µg of proteins. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.

Hyaluronic acid level in NHDFs

Finally, the dermal HA levels have been measured. Under our experimental conditions, the new NSM at 0.03% and 0.1% significantly ($p<0.01$) increased level of HA in NHDFs in a dose-dependent manner (*Fig.-3*). The above results reveal that this novel natural skin moisturizer may act on key moisturizing factors in different cutaneous layers to play a triple skin hydration.

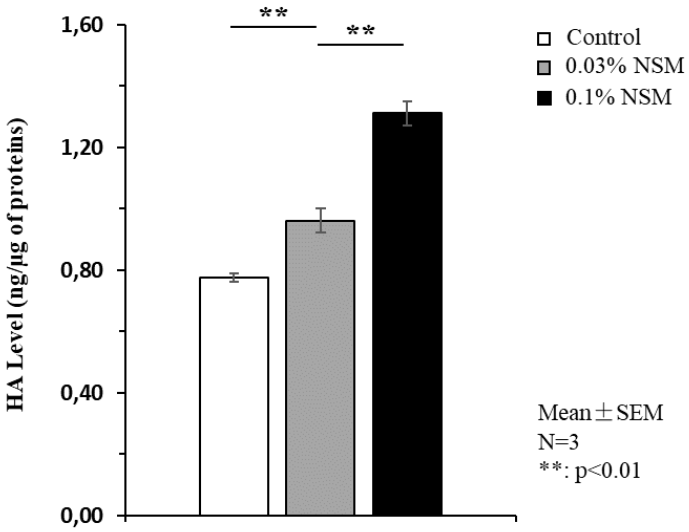

Fig.-3 Effect of the new NSM on HA production in NHDFs.
Results are expressed as ng of hyaluronic acid per µg of proteins. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.

Discussion

Climate change and population diversity contribute to the complexities of dry skin conditions. Each skin layer requires a tailored hydration strategy in order to maintain the specific water balance, which is essential for skin cell and barrier function. As the outermost layer of the epidermis, SC is thought to perform several important protective cutaneous functions [5]. The water content of SC is required for corneocyte maturation and skin desquamation [6]. As one of the main factors in maintaining SC water balance, NMF plays a central role in skin barrier hydration. The stimulation of epidermal production of NMF is considered an important strategy for hydration homeostasis in the upper skin. Recent evidence suggests that reduced BLMH expression results in a low hydration state of dry skin [1], and human epidermal BLMH may also be involved in wound healing as well as secretion of pro-inflammatory cytokines [11]. BLMH as a critical protease required for the complete

degradation of filaggrin fragments into free amino acids, can be a key target for cutaneous surface moisturization and barrier protection.

For many dry skin conditions, keratinocytes may be exposed to hyperosmotic stress. Intracellular accumulation of taurine protects keratinocytes from osmotically and UV-induced damages by maintaining cellular water balance and survival [12]. Taurine has also been shown to have antioxidant, anti-inflammatory and membrane stabilizing activities [13, 14,]. Therefore, taurine can be a key target of epidermal cell hydration homeostasis in skin dryness. An age-related decline in taurine was recently found in rodent skin [15], whether the same trend occurs in human skin requires further investigation. Dermal HA plays a multifaceted role in modulating various biological processes, its water holding ability results in softer, smoother and more radiant skin. Exogenous HA is widely used in epidermal moisturizing and anti-aging, while stimulating the production of endogenous HA is of great significance for deep skin hydration.

In this work, we showed the coordinated up-regulation of three key hydration factors targeting different cutaneous layers. These have been confirmed in clinical trials (data not shown). Interestingly, in addition to maintaining skin water balance, these factors both have multiple biological activities, especially anti-inflammatory. In the meanwhile, their expression and bioactivities appear to be inversely correlated with age. These seem to confirm the important link between skin moisture loss, immune barrier function and aging.

Conclusion

Each skin layer requires a specific hydration strategy in order to maintain overall skin water balance. BLMH is considered to be essential for the complete degradation of filaggrin into free amino acids, the latter act as NMF in the upper stratum corneum. BLMH is critical for skin barrier function and upper skin hydration. For many dry skin conditions, keratinocytes may be exposed to hyperosmotic stress. Intracellular accumulation of taurine protects keratinocytes from osmotically and UV-induced damages by maintaining cellular water balance and survival. Dermal HA plays a multifaceted role in modulating various biological processes, its water holding ability results in softer, smoother and more radiant skin. The coordinated increase in epidermal BLMH biosynthesis, intracellular taurine

accumulation and dermal HA production address multi-layered hydration. This trio strategy may represent a novel approach for maintaining skin hydration homeostasis.

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Conflict of Interest Statement

There is no any conflict of interest by the authors competing interests.

References

1. Son ED, Kim Y, et al (2015) Skin dryness in apparently healthy human skin is associated with decreased expression of bleomycin hydrolase in the stratum corneum. Clin Exp Dermatol. 40(3):247–253.
2. Kamata Y, Taniguchi A, et al (2009) Neutral cysteine protease bleomycin hydrolase is essential for the breakdown of deiminated filaggrin into amino acids. J Biol Chem. 284(19):12829–12836.
3. Jokura Y, Ishikawa S, et al (1995) Molecular analysis of elastic properties of the stratum corneum by solid state ^{13}C-nuclear magnetic resonance spectroscopy. J Invest Dermatol. 104:806–812.
4. Rawlings AV, Harding CR (2004) Moisturization and skin barrier function. Dermatol Ther. 17 Suppl 1:43–48.
5. Elias PM (2005) Stratum corneum defensive functions: an integrated view. J Invest Dermatol. 125(2):183–200.
6. Verdier-Sévrain S, Bonté F (2007) Skin hydration: a review on its molecular mechanisms. J Cosmet Dermatol. 6(2):75–82.
7. EL-Chami C, Haslam IS, et al (2014) Role of organic osmolytes in water homoeostasis in skin. Exp Dermatol. 23(8):534–537.
8. Janeke G, Siefken W, et al (2003) Role of taurine accumulation in keratinocyte hydration. J Invest Dermatol. 121(2):354–361.

9. Necas J, Bartosicova L, et al (2008) Hyaluronic acid. (hyaluronan): a review. Vet Med. 8:397–411.
10. Masson F (2010) Skin hydration and hyaluronic acid. Ann Dermatol Venereol. 137 Suppl 1:S23–25.
11. Riise R, Odqvist L, et al (2019) Bleomycin hydrolase regulates the release of chemokines important for inflammation and wound healing by keratinocytes. Sci Rep. 9:20407.
12. Warskulat U, Reinen A, et al (2004) The osmolyte strategy of normal human keratinocytes in maintaining cell homeostasis. J Invest Dermatol. 123:516–521.
13. Schaffer S, Takahashi K, Azuma J (2000) Role of osmoregulation in the actions of taurine. Amino Acids 19:527–546.
14. Schaffer S, Azuma J, et al (2003) Why is taurine cytoprotective? Adv Exp Med Biol. 526:307–321.
15. Yoshimura T, Inokuchi Y, et al (2021) Age-related decline in the taurine content of the skin in rodents. Amino Acids 53: 429–434.