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"Hard Water Makes Hair and Scalp Barrier Function Weaker ~ To protect hair and scalp from silent damage ~"

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1. Introduction

In Japan, the water for daily use is soft water, which is used daily to wash hair. It is generally known that when hair is exposed to hard water in regions outside of Japan where the water for daily use is hard water, or when hair is exposed to seawater, hot springs, etc., the texture and appearance of the hair deteriorate and do not recover for a certain period of time. Also, with regard to the scalp, hard water is said to cause dandruff and itching. These are considered problematic by the general public. Soft water is water with low concentrations of Ca^{2+} and Mg^{2+}, while hard water is water with high concentrations of these elements. The World Health Organization (WHO) defines soft water as water with a concentration of less than 120 mg/L and hard water as water with a concentration of 120 mg/L or more. It is well known that hard water generally inhibits the foaming of anionic surfactants in shampoos, and research has been conducted on the types and combinations of anionic surfactants that produce foam even in hard water [1]. However, the effects of hard water do not stop there. Exposure of hair to hard water results in an unruly, dry appearance and rough texture, which is attributed to the loss of smoothness due to the deposition of metallic salts on the hair surface [2,3].

Furthermore, in our previous study, we found significant fading of hair color in hard water, a decrease in tensile strength, and a change in the isoelectric point of the hair [4]. We thought that hard water may have a persistent effect not only on the top surface of the hair but also on the inside of the hair.

In this study, we hypothesized that these phenomena are caused by the cleavage of ionic bonds between keratin fibers in hard water. We further investigated the effects of hard water on hair and skin, which contain similar keratin fibers, and explored ways to inhibit or prevent these phenomena.

2. Materials and Methods

Preparation of soft and hard water

Commercial hard water was diluted with distilled water to obtain soft water with a hardness of 20 mg/L and hard water with a hardness of 400 mg/L (Table 1.).

Table 1. Main ion contents of soft and hard water used in the experiment

Ion	Soft water	Hard water
Calcium	6.344	126.886
Magnesium	1.010	20.199
Potassium	0.038	0.758
Hardness (mg/L)	**20**	**400**

Determination of eluted fatty acids from hair

0.3 g of black Asian hair (BS-B3N; Beaulax) was washed and soaked in 30 mL of 1% alkyl glucoside dissolved in soft or hard water and allowed to stand at 40 °C for 1 hour. The hair was removed and the extract was obtained. The extract was concentrated, and 0.01 mg ethyl heptadecanoate (as hexane solution) and 50 µL of 1.25 mol/L HCl/MeOH was added, and the ester exchange reaction was carried out at 80 °C for 30 minutes. In addition, 100 µL of distilled water and 300 µL of hexane were added, and after stirring, the hexane layer was analyzed by GC-MS. From the obtained peak area, the amount of eluted fatty acids was calculated as an approximation using the peak of C17 fatty acid with known concentration as a standard.

Evaluation of ingredient permeability using keratin film

To evaluate the ingredient permeability of keratin fibers, keratin films derived from hair were used. 138 mg/mL rhodamine isothiocyanate (RITC; R1755, SIGMA-ALDRICH) was added and allowed to stand at 40 °C for 1 hour. Then, they were washed with distilled water and sections of 16 µm thickness were prepared with a microtome (SM2000R, LEICA). The sections were observed under a fluorescence microscope (BZ-X810, KEYENCE).

Evaluation of skin irritation of ingredients using a 3D cultured epidermal model

Using a 3D cultured epidermal model (EPI-MODEL24; LabCyte), the skin irritancy of ingredients in soft and hard water was evaluated using cell viability as an indicator. Benzalkonium chloride was used as the irritant. The 3D cultured epidermal models were pre-cultured in a CO_2 incubator (5%, 37 °C) for 15-30 hours, then the test samples were added. The models were cultured in a CO_2 incubator (5%, 37 °C) for 15 minutes, and washed 10 times with PBS. Then, the models were post-cultured in a CO_2 incubator (5%, 37 °C) for 42 hours and cell viability was calculated by MTT assay.

Reduction and prevention tests for effects caused by hard water

The ability of pentasodium pentetate and fulvic acid to reduce or prevent the effects of hard water was tested. For permeability of the ingredients to the keratin film, the skin was treated with 0.05% pentasodium pentetate or fulvic acid at 40 °C for 1 hour, either simultaneously with or before the addition of RITC. For skin irritation using a 3D cultured epidermal model, the models were exposed to 0.05% or 1% pentasodium pentetate or fulvic acid dissolved in hard water for 2 hours in a CO_2 incubator (5%, 37 °C) after pre-culture, washed, and exposed to the test samples.

3. Results

Components leakage from the hair increases in hard water.

Exposure of hair to nonionic surfactants in hard water increased the elution of each fatty acid compared to soft water (Fig. 1). The changes in the amount of each fatty acid eluted varied greatly depending on the damage treatment, elution time, and other conditions (data not shown).

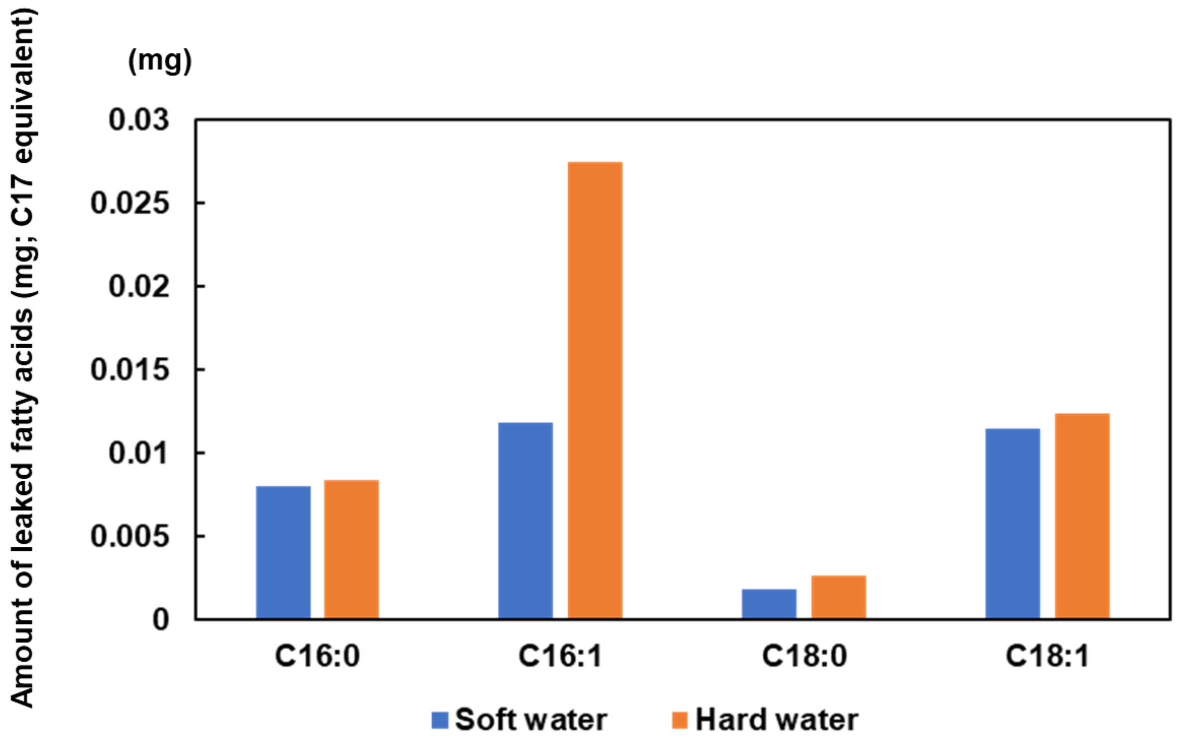

Figure 1. Leakage of hair fatty acids in soft and hard water

Lipids were eluted from hair with 1% alkyl glucoside in soft and hard water and analyzed by GC-MS. The peak areas were converted to the concentration by comparison to the peak area of C17 fatty acids with known concentration added to the samples. The results showed an increase in fatty acid elution in hard water compared to soft water.

The ingredient permeability of keratin fibers increases in hard water.

The permeation of fluorescent dyes was evaluated using keratin films. As a result, the permeation of the ingredients into the keratin film was increased in hard water compared to soft water (Fig. 2).

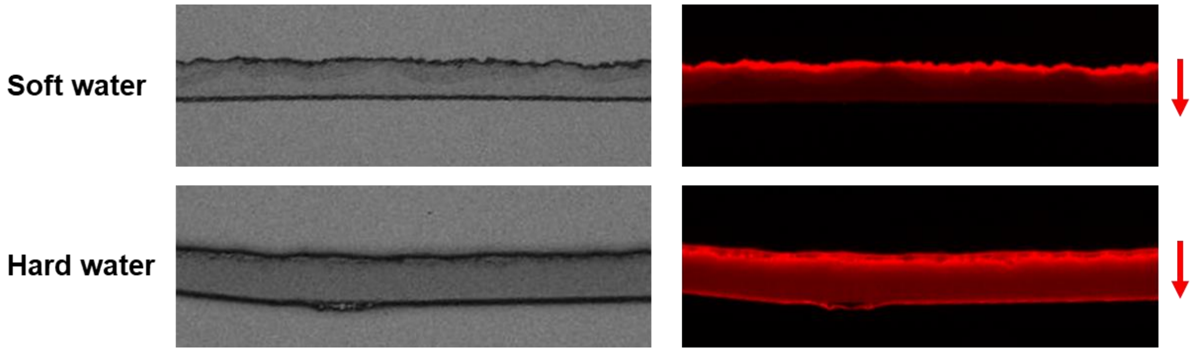

Figure 2. ingredient permeability of keratin fibers in soft and hard water
The fluorescent dye RITC was used to check the permeability of ingredients to keratin film in soft and hard water. Arrows indicate the direction of RITC penetration. The results showed that RITC penetration increased in hard water compared to soft water.

<u>Hard water increases skin irritation due to ingredients.</u>

The irritation to 3D cultured epidermal models caused by benzalkonium chloride at various concentrations in soft or hard water was quantified using cell viability as an indicator. The results showed that benzalkonium chloride increased skin irritation in hard water compared to soft water (Fig. 3).

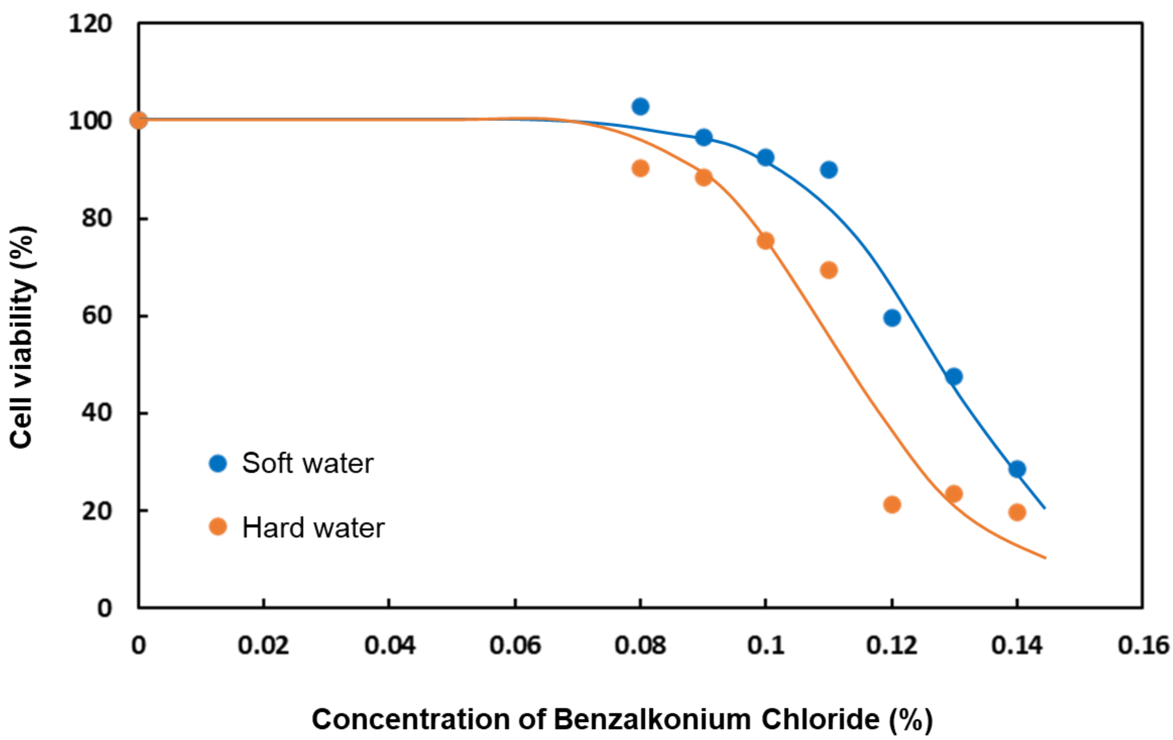

Figure 3. Irritation to 3D cultured epidermal models by irritants in soft and hard water
We investigated whether the irritancy of benzalkonium chloride changes in soft and hard water using a 3D cultured epidermal model. The results showed that the irritancy of benzalkonium chloride increased in hard water compared to soft water.

Permeation of ingredients into the keratin film in hard water is reduced by fulvic acid.

Keratin films treated with fulvic acid, simultaneously with RITC exposure, showed less permeation of fluorescent dye than those without fulvic acid treatment. Similarly, pretreatment with fulvic acid before exposure to RITC reduced the permeation of the fluorescent dye. Pentasodium pentetate also showed some permeation reduction by simultaneous treatment with RITC, but the effect was less pronounced than with fulvic acid under the same conditions (Fig. 4).

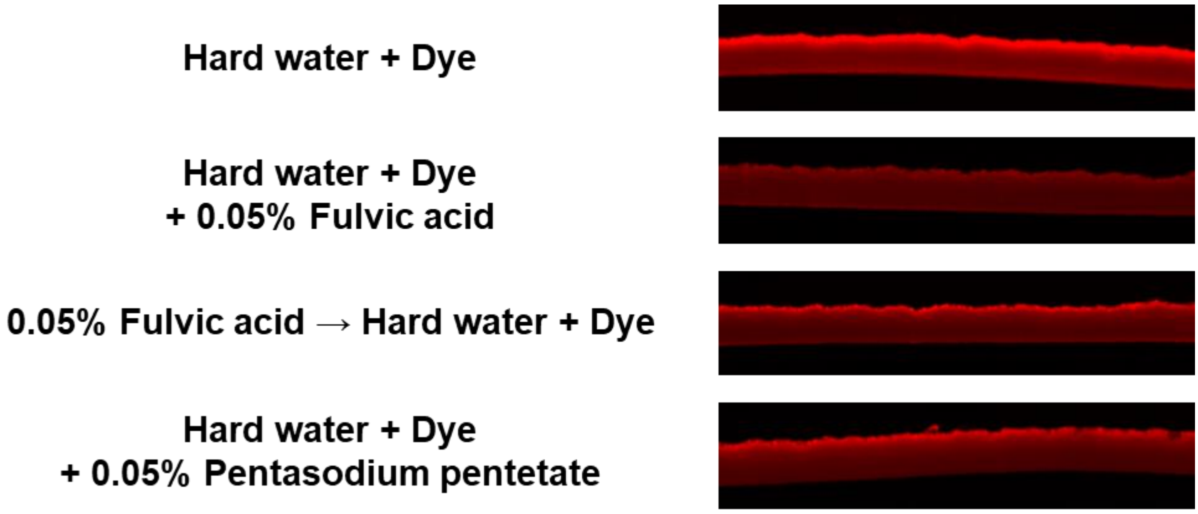

Figure 4. Reduction of ingredient penetration into keratin film by fulvic acid

We checked whether the penetration of RITC into keratin film in hard water could be reduced by fulvic acid and pentasodium pentetate. The results showed that treatment with fulvic acid simultaneously with RITC strongly reduced the penetration of RITC (second from the top). Fulvic acid as a pretreatment for RITC exposure was also found to be effective (third from top). Treatment with pentasodium pentetate was also effective, but the effect of simultaneous treatment with RITC was comparable to the effect of the pretreatment of fulvic acid (bottom).

Fulvic acid pretreatment reduces irritation of 3D cultured epidermal models in hard water.

Cell viability was increased in 3D cultured epidermal models pretreated with fulvic acid before exposure to benzalkonium chloride compared to those without fulvic acid. The concentration of fulvic acid used for pretreatment was more effective at 1% than at 0.05%. Conversely, pretreatment with 1% pentasodium pentetate reduced cell viability (Fig. 5).

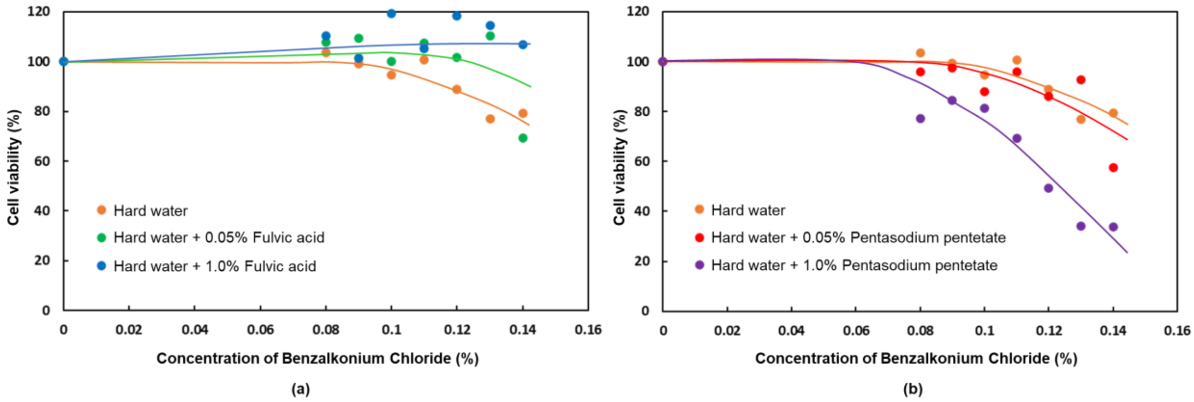

Figure 5. Fulvic acid pretreatment prevents irritation to 3D cultured epidermal models in hard water

The effects of fulvic acid and pentasodium pentetate on the irritancy of benzalkonium chloride in hard water were confirmed using a 3D cultured epidermal model. (a) Pretreatment with fulvic acid before exposure to benzalkonium chloride increased cell viability in a concentration-dependent manner. (b) In contrast, pretreatment with 5Na pentetate showed no inhibitory effect on the irritation caused by benzalkonium chloride , and conversely resulted in a decrease in cell viability.

4. Discussion

In our study, we found that hard water increases leakage of not only exogenous ingredients such as hair color dyes but also hair-derived components such as fatty acids. This result is consistent with our hypothesis that the binding of metal ions in hard water to acidic functional groups such as carboxyl groups in hair fibers causes a decrease in ionic bonding between hair fibers, resulting in fragility of hair and easy leaking of components. To confirm this, we first tested the ingredient permeability of the keratin film. Considering that keratin film does not have cell membrane complex and is suitable for confirming the permeability of hydrophilic components, and that anionic components may bind to metal ions and significantly change the properties of the molecules, we used RITC, a hydrophilic, cationic fluorescent dye, as the ingredient to check penetration. As a result, hard water increased the permeation of ingredients into the keratin film. It has also been reported that dye leakage from dyed keratin films is increased by certain metal ions [5]. These findings suggest that metal ions in hard water may affect the interactions between keratin fibers or between keratin and keratin-associated protein, resulting in increasing ingredient permeability of the hair fiber.

Furthermore, we focused on the stratum corneum, which is composed mainly of aggregates of acidic and basic keratins, like hair. The barrier function of the stratum corneum of the skin has the important function of protecting the skin by inhibiting the penetration of ingredients from the outside. If hard water affects this function, it could be the cause of the deterioration of the scalp condition due to hard water as described by the general public. Specifically, we hypothesized that metal ions in hard water would weaken interactions between keratins in the stratum corneum of the skin and increase ingredient permeability, thereby elevating skin irritation caused by irritants. And to confirm this, we tested the changes in skin irritation caused by the irritants on 3D cultured epidermal models in soft or hard water. We used benzalkonium chloride, which is hydrophilic, cationic, and frequently used in cosmetics, as the irritant. And the results showed that cell viability after benzalkonium chloride exposure was lower in hard water than in soft water. This suggests that skin irritation due to irritants is elevated in hard water. In other words, washing hair in hard water not only increases lipid and hair color dye leakage from the hair, but also risks exacerbating scalp irritation from the irritants in shampoo or other products.

We confirmed whether pentasodium pentetate and fulvic acid could inhibit the effects of hard water on hair and scalp. As a result, a higher inhibitory effect on ingredient permeability of keratin film was shown with fulvic acid than with pentasodium pentetate. Interestingly, not only is fulvic acid effective when treated with fluorescent dyes simultaneously, but also the pretreatment can prevent the penetration of fluorescent dyes by hard water in a preventive manner. It is speculated that the residual fulvic acid in the keratin fibers efficiently removes metal ions before they penetrate the keratin fibers upon subsequent exposure to hard water. Similar results were obtained from experiments using 3D cultured epidermal models. Fulvic acid showed a concentration dependent inhibitory effect on irritation by benzalkonium chloride, while pentasodium pentetate was less effective at low concentrations and conversely enhanced irritation at higher concentrations. This is thought to be the result of the cellular irritancy of pentasodium pentetate being added to the irritation of benzalkonium chloride. In contrast, fulvic acid was found to exhibit no cellular irritation even at relatively high concentrations and to inhibit the promotion of ingredient permeability of keratin fibers by hard water.

5. Conclusion

Our study has shown that hard water increases the ingredient permeation of keratin fibers and increases ingredient leakage and ingress. These effects are thought to be causes of the poor condition of hair and scalp and the persistence of the condition, in hard water environments. We also found an ingredient that counteracts these effects of hard water. It is hoped that this will lead to the development of technology to protect the hair and scalp from the effects of hard water quality, either symptomatically or prophylactically.

6. Reference

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