Acta Derm Venereol 2015; 95: 787–791

INVESTIGATIVE REPORT

Ultra-pure Soft Water Ameliorates Atopic Skin Disease by Preventing Metallic Soap Deposition in NC/Tnd Mice and Reduces Skin Dryness in Humans

Akane TANAKA[1,2], Akira MATSUDA[1], Kyungsook JUNG[1], Hyosun JANG[2], Ginnae AHN[1], Saori ISHIZAKA[2], Yosuke AMAGAI[2], Kumiko OIDA[2], Peter D. ARKWRIGHT[3] and Hiroshi MATSUDA[1,2]

[1]Comparative Animal Medicine and Veterinary Molecular Pathology and Therapeutics, Division of Animal Life Science, Institute of Agriculture, [2]Cooperative Major in Advanced Health Science, Graduate School of Bio-Applications and System Engineering, Tokyo University of Agriculture and Technology, Tokyo, Japan, and [3]Department of Paediatric Allergy and Immunology, University of Manchester, Royal Manchester Children's Hospital, Manchester, UK

Mineral ions in tap water react with fatty acids in soap, leading to the formation of insoluble precipitate (metallic soap) on skin during washing. We hypothesised that metallic soap might negatively alter skin conditions. Application of metallic soap onto the skin of NC/Tnd mice with allergic dermatitis further induced inflammation with elevation of plasma immunoglobulin E and proinflammatory cytokine expression. Pruritus and dryness were ameliorated when the back of mice was washed with soap in Ca^{2+}- and Mg^{2+}-free ultra-pure soft water (UPSW). Washing in UPSW, but not tap water, also protected the skin of healthy volunteers from the soap deposition. Furthermore, 4 weeks of showering with UPSW reduced dryness and pruritus of human subjects with dry skin. Washing with UPSW may be therapeutically beneficial in patients with skin troubles. ***Key words: atopic dermatitis; animal model; metallic soap; skin barrier; pruritus.***

Accepted Mar 2, 2015; Epub ahead of print Mar 5, 2015

Acta Derm Venereol 2015; 95: 787–791.

Akane Tanaka and Hiroshi Matsuda, Laboratories of Comparative Animal Medicine and Veterinary Molecular Pathology and Therapeutics, Division of Animal Life Science, Institute of Agriculture, Tokyo University of Agriculture and Technology, 3-8-5 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan. E-mails: akane@cc.tuat.ac.jp, hiro@cc.tuat.ac.jp

Susceptibility to atopic dermatitis (AD) is associated with both genetic and environmental factors, which disrupt skin barrier function (1, 2). Profilaggrin produced by keratinocytes is known to be a key factor in maintaining skin barrier function. Filaggrin gene (*FLG*) mutations as well as irritants and allergens that stimulate inflammatory cytokine responses may both reduce filaggrin expression in the epidermis (3, 4). Thus, impaired skin barrier function facilitates penetration of irritants and allergens, which promotes a vicious cycle of skin barrier dysfunction and inflammation (5–7).

Even water, particularly hard water, which is characterised by a high content of multivalent cations such as Ca^{2+} and Mg^{2+}, has been reported to exacerbate AD through the formation of metallic soap, which disrupts the skin barrier function (8–10). Mineral salts in water react with fatty acids of soap to form an insoluble precipitate known as metallic soap (11, 12). Preventing the disruption of the skin barrier by metallic soaps might be expected to ameliorate the symptoms of AD. However, a previously published randomised, observer-blinded trial using ion-exchange water softeners failed to demonstrate any beneficial effect in 336 children with moderate to severe AD (13, 14). The ion-exchanger used in the study was a synthetic polystyrene resin, which removed Ca^{2+} (and Mg^{2+}) from household water to less than 20 mg $CaCO_3$/l. We previously examined the effects of ultra-pure soft water (UPSW), without detectable Ca^{2+} and Mg^{2+} (< 1 mg/l) on spontaneous AD in dogs (15, 16). Compared with use of a cleanser in hard water, shampoo treatment with UPSW improved pruritus and dermatitis in dogs, without any adverse events (15). In the present study, we evaluated the effects of UPSW on the skin of NC/Tnd mice, an animal model of AD (17), both with regards to clinical symptoms, and to skin barrier function, as measured by transepidermal water loss (TEWL). To investigate the underlying mechanism, metallic soap was applied onto the tape-stripped mouse skin, and initiation of allergic inflammation including plasma total IgE and proinflammatory cytokines were evaluated. Finally, in a study of human volunteers with dry and itchy skin, we evaluated beneficial effects of showering with UPSW on their skin conditions. This is the first report to show the induction of allergic responses by topical metallic soap on the barrier-impaired animal skin, as well as the beneficial effects of UPSW in human adults with dry and itchy skin.

MATERIALS AND METHODS (See Appendix S1[1])

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2083

RESULTS

Suppression of dermatitis in NC/Tnd mice

To examine the *in vivo* effect of UPSW in NC/Tnd mice with active dermatitis, the clipped dorsal skin was washed with soap and rinsed with pre-warmed (37–40ºC) tap water or UPSW for 3 min once a day for 3 weeks. As shown in Fig. 1a, the dermatitis severity scores gradually decreased in mice treated with soap and UPSW compared with control mice and mice treated with soap and tap water, reaching statistical significance after 16 days. Fig. 1b shows that while the scratching behaviour of mice worsened after washing with soap and tap water (and in the control group), treatment with soap and UPSW suppressed the scratching events. Furthermore, TEWL was significantly lower after washing with soap and UPSW compared to soap and tap water (Fig. 1c).

Proinflammatory effect of metallic soap in barrier-disrupted skin

To further explore the negative effects of soap and tap water, we examined the proinflammatory properties of metallic soap. In this study, we used NC/Tnd mice in a specific pathogen-free (SPF) condition where AD did not developed. After tape stripping 3 times (TEWL 10–20 g/h/m^2), metallic soap was applied to the barrier-disrupted skin twice a week for 4 weeks (see Appendix S1[1]). Plasma IgE concentrations in NC/Tnd mice subjected to metallic soap application were increased significantly after 4 weeks, even in mice maintained under SPF conditions compared to those in controls applied with diluent or soap alone (Fig. 2a). Topical application of metallic soap generated with Ca^{2+} and to a lesser extent with Mg^{2+}, induced IgE production, whereas there was no increase in IgE levels in mice treated with saline diluent or soap alone.

Skin histology showed that metallic soap containing Ca^{2+} or Mg^{2+} induced epidermal hyperplasia and an inflammatory dermal infiltrate in NC/Tnd mice in contrast to mice treated with diluent or non-metallic soap (Fig. 2b).

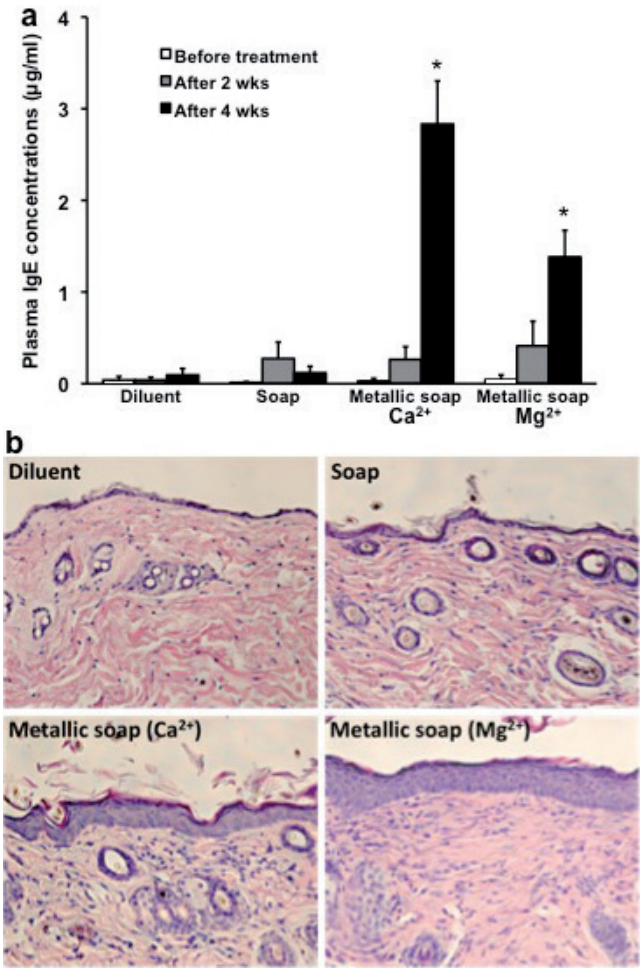

Fig. 2. Effects of topical metallic soap application onto the barrier disrupted skin of NC/Tnd mice. (a) Plasma total IgE concentrations in NC/Tnd mice (12 mice in each group) in which soap, or metallic soap had been repeatedly applied to the skin for up to 4 weeks. Saline was used as diluent (*$p < 0.05$, compared with diluent-treated group). (b) Skin histology of NC/Tnd mice (8 mice in each group) in which soap, or metallic soap had repeatedly been applied for 8 weeks. Saline was used as diluent. Haematoxylin and eosin staining shows hyperplasia of epidermis with dermal inflammatory infiltrate, particularly when calcium-containing metallic soap was used.

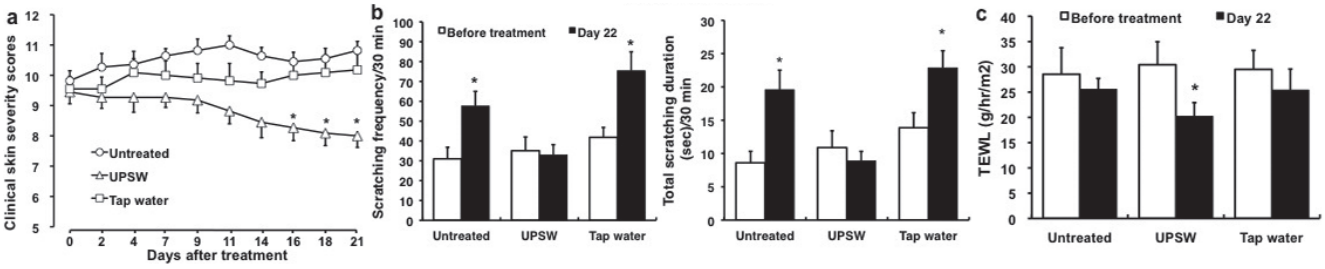

Fig. 1. Changes in clinical symptoms of NC/Tnd mice with atopic dermatitis (AD) treated with either washing the skin with soap and tap water, or with soap and ultra-pure soft water (UPSW), when compared with no washing. (a) Clinical mean severity scores (14 mice in each group; *$p < 0.05$ compared with mice treated with tap water). (b) Scratching frequency (left) and duration (right) over 30 min in NC/Tnd mice ($n = 14$) at baseline and 22 days after washing skin with soap and UPSW or tap water compared with untreated controls. Scratching behaviour was measured using a SCLABA®-Real system (*$p < 0.05$ compared with pre-treatment). (c) TEWL of dorsal area in NC/Tnd mice ($n = 14$) at baseline and 22 days after washing (same as in b).

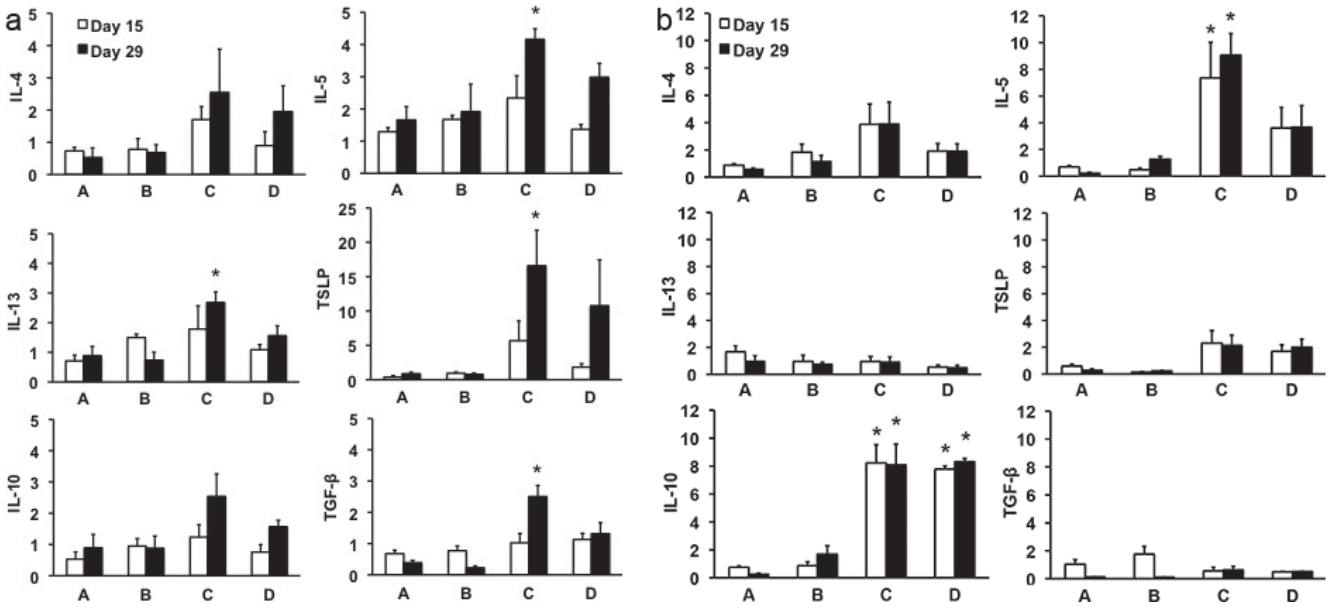

Fig. 3. Relative expression of proinflammatory cytokines (interleukin (IL)-4, -5, -10, -13, TSLP, TGF-β) as measured by PCR in (a) the dorsal skin and (b) axillary lymph nodes of NC/Tnd mice. Relative expression levels of mice applied with non-metallic soap (B), metallic soap with Ca^{2+} (C), or metallic soap with Mg^{2+} (D) were compared with diluent (A). $*p < 0.05$, when compared with the value in diluent-treated group. PCR reaction was performed with triplication. All data are represented as mean ± SE of 12 mice in each group.

Gene expression of IL-4, IL-5, IL-13, TSLP, and TGF-β as measured by real-time PCR were up-regulated in the skin of mice treated with Ca^{2+} and Mg^{2+} metallic soaps for 4 weeks (Fig. 3a). No significant changes in IFN-γ expression were detected (data not shown). The skin of mice treated with diluent or soap alone did not show increased expression of these cytokines. IL-4, IL-5, and IL-10 levels were also increased in the regional lymph nodes of mice treated with topical metallic soap compared with controls, whereas IFN-γ, TGF-β, TSLP, and IL-13 levels remained unchanged (Fig. 3b).

Effect of ultra-pure soft water on the metallic soap content of the stratum corneum in healthy volunteers

As metal ions in tap water form an insoluble precipitate with fatty acids in soaps, deposition of metallic soap on the skin can be estimated by measuring residual fatty acids. We examined fatty acid residues in the stratum corneum of 10 healthy volunteers who had rinsed their skin from soap, either with tap water or UPSW for 1–3 min. Stratum corneum was collected by tape-stripping at baseline, 60, 120 and 180 s. The amount of lauric acid residue was measured by gas chromatography. When the skin was rinsed with UPSW, the lauric acid was almost completely gone by 90 s, while even after 180 s of washing with tap water over 80% of the fatty acid remained in the stratum corneum (Fig. 4a). We also checked dependency on $CaCO_3$ concentrations in water. As indicated in Fig. 4b, the harder the water the more fatty acid of the soap remained on the skin after rinsing for 90 s.

Effect of ultra-pure soft water on skin symptoms and TEWL of volunteers with the dry and itchy skin

The clinical skin scores were decreased significantly in the adult volunteers after 2 and 4 weeks of daily showering compared to before treatment (Fig. 4c). Indeed by 29 days, pruritus almost completely resolved in this cohort of 8 females with mild AD and dry skin. Showering with UPSW also significantly increased the water content of the stratum corneum from a median of 21 to 30 arbitrary units (a.u.) (Fig. 4d). This was associated with a significant reduction in TEWL at 29 days compared to both before and after 15 days of UPSW treatment (Fig. 4d).

DISCUSSION

In the current study, we explored the effects of Ca^{2+} and Mg^{2+} in tap water on barrier-damaged skin using $CaCO_3$-free UPSW. A previous study indicated that lamellar body secretion from skin keratinocytes was regulated by the epidermal extracellular Ca^{2+} gradient (23). Hypothetically, showering with UPSW via reducing extracellular Ca^{2+} levels might accelerate lamellar body secretion and hence promote skin barrier recovery. Conversely, tap water containing metal ions, which react with fatty acids in soap to form metallic soap, may perturb the skin barrier (11, 12). Indeed, we demonstrated that metallic soap can induce and exacerbate AD in the NC/Tnd mouse model, whereas UPSW had the opposite effect. Furthermore, rinsing human skin with UPSW effectively removed metallic

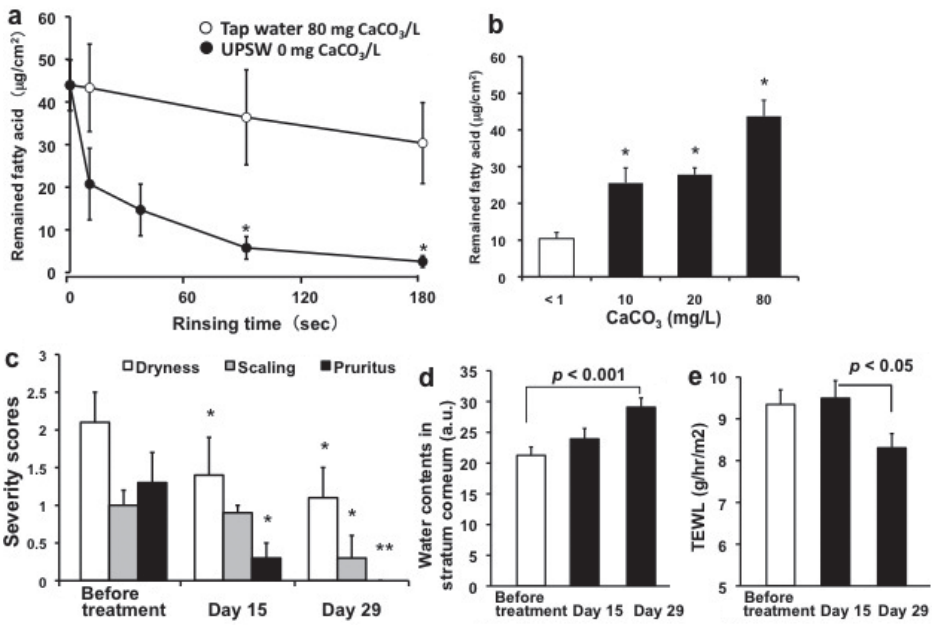

Fig. 4. Changes in skin conditions and skin barrier function in human volunteers with dry skin having daily showers with ultra-pure soft water (UPSW) for 29 days when compared with baseline. (a) Lauric acid contents in stratum corneum of the forearms of 6 human healthy volunteers rinsed with UPSW or tap water for indicated sec were measured by gas chromatography (*$p < 0.05$ compared with subjects rinsed with tap water). (b) Lauric acid contents in stratum corneum of the forearms of 6 human healthy volunteers rinsed in water with various concentrations of $CaCO_3$ for 90 s were measured by gas chromatography (*$p < 0.05$ compared with subjects rinsed with UPSW (< 1 mg $CaCO_3$/l)). (c–e) Effect of daily UPSW shower for 4 weeks in 8 volunteers who were diagnosed as mild atopic dermatitis. (c) Clinical scores in terms of dryness (white column), scaling (gray column) and pruritus scored (black column) *$p < 0.05$, **$p < 0.01$. (d) Skin water content measured using a corneometer (indicated by arbitrary units a.u.). (e) TEWL measured on the forearms.

soap from the stratum corneum and improved dermatitis and pruritus scores in patients with mild AD. In contrast, washing with tap water led to no improvement in clinical severity.

Because metallic soap easily remain in the skin, it may act as an irritant and might even induce allergic dermatitis. The higher the concentration of Ca^{2+} and Mg^{2+}, the more soap is needed to lather the water thus creating a vicious circle, especially since the skin barrier can also be disrupted by scrubbing the skin with a sponge. The use of UPSW, on the other hand, reduces the risks associated with soap washing.

In the NC/Tnd mouse model, we also demonstrated that the clinical benefit of UPSW is mirrored by an improvement in TEWL and a reduction in plasma IgE and Th2 cytokine concentration. In barrier-disrupted skin, epidermal Langerhans cells elongate their dendrites through tight junctions in the keratinocyte layer, and take up external antigens, thereby associating with the initiation of allergic responses (24). Even a weak external stimuli may evoke recruitment of Langerhans cells around hair follicles and trigger inflammation (25). Our results allow us to speculate that destruction of the skin barrier by metallic soap may activate the elongation of dendrites and facilitate initiation of allergic inflammation.

Previous epidemiological studies provided some evidence of a relationship between water hardness and the prevalence of AD (8–10). This was first demonstrated in 1998 in a study of 4,141 primary school children in the UK (8). A multicentre, randomised, controlled trial was set up in the UK in 2008 to study the effects of ion-exchange water softeners in the treatment of eczema in children (11). Although significant differences were found in some secondary outcomes as reported by parents, no benefit was seen regarding primary clinical scores in the children (12). Using the ion-exchanger, the authors stated that the water hardness was reduced to < 20 mg $CaCO_3$/l. As seen in Fig. 4b, metallic soap can be generated even in water containing low concentrations of metallic ions, which is a possible explanation for the lack of efficacy in the previous trial, compared with the significant improvement seen in our study which used UPSW with < 1 mg $CaCO_3$/l. In fact, we have recently demonstrated a beneficial effect of UPSW on barrier function in children with AD (26). A larger, multicentre study is needed, together with studies of the effects of metallic soaps on cytokine production, particularly TSLP and other Th2-promoting cytokines in human skin keratinocytes.

ACKNOWLEDGEMENTS

We thank Dr Simon G Danby (Department of Infection & Immunity, Faculty of Medicine, Dentistry and Health, The University of Sheffield) for reviewing our manuscript. We appreciate Dr. Masaki Takai (R&D Center, Miura Co., Ltd.) for providing the cation-exchange UPSW generator. This work was supported by Grant-in-Aid for Scientific Research on Priority Areas A (No. 24248055) and Areas B (No. 24380168) provided by the Japan Society for the Promotion of Science, Japan.

The authors declare no conflict of interest.

REFERENCES

1. De Benedetto A, Kubo A, Beck LA. Skin barrier disruption: a requirement for allergen sensitization? J Invest Dermatol 2012; 132: 949–963.
2. Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME,

Moustafa M, et al. Epidermal barrier dysfunction in atopic dermatitis. J Invest Dermatol 2009; 129: 1892–1908.
3. McAleer MA, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. J Allergy Clin Immunol 2013; 131: 280–291.
4. Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. N Engl J Med 2011; 365: 1315–1327.
5. Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Mechin MC, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. J Allergy Clin Immunol 2013; 131: 1094–1102.
6. Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol 2009; 124: R7–R12.
7. Gittler JK, Krueger JG, Guttman-Yassky E. Atopic dermatitis results in intrinsic barrier and immune abnormalities: implications for contact dermatitis. J Allergy Clin Immunol 2013; 131: 300–313.
8. McNally NJ, Williams HC, Phillips DR, Smallman-Raynor M, Lewis S, Venn A, Britton J. Atopic eczema and domestic water hardness. Lancet 1998; 352: 527–531.
9. Miyake Y, Yokoyama T, Yura A, Iki M, Shimizu T. Ecological association of water hardness with prevalence of childhood atopic dermatitis in a Japanese urban area. Environ Res 2004; 94: 33–37.
10. Chaumont A, Voisin C, Sardella A, Bernard A. Interactions between domestic water hardness, infant swimming and atopy in the development of childhood eczema. Environ Res 2012; 116: 52–57.
11. Friedman M, Wolf R. Chemistry of soaps and detergents: various types of commercial products and their ingredients. Clin Dermatol 1996; 14: 7–13.
12. Warren R, Ertel KD, Bartolo RG, Levine MJ, Bryant PB, Wong LF. The influence of hard water (calcium) and surfactants on irritant contact dermatitis. Contact Dermatitis 1996; 35: 337–343.
13. Thomas KS, Sach TH; SWET Trial Investigators. A multicentre randomized controlled trial of ion-exchange water softeners for the treatment of eczema in children: protocol for the Softened Water Eczema Trial (SWET) (ISRCTN: 71423189). Br J Dermatol 2008; 159: 561–566.
14. Thomas KS, Dean T, O'Leary C, Sach TH, Koller K, Frost A, Williams HC; SWET Trial Team. A randomised controlled trial of ion-exchange water softeners for the treatment of eczema in children. PLoS Med 2011; 8: e1000395.
15. Ohmori K, Tanaka A, Makita Y, Takai M, Yoshinari Y, Matsuda H. Pilot evaluation of the efficacy of shampoo treatment with ultrapure soft water for canine pruritus. Vet Dermatol 2010; 21: 477–483.
16. Olivry T. What can dogs bring to atopic dermatitis research? Chem Immunol Allergy 2012; 96: 61–72.
17. Tanaka A, Amaqai Y, Oida K, Matsuda H. Recent findings in mouse models for human atopic dermatitis. Exp Anim 2012; 61: 77–84.
18. Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, et al. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int Immunol 1997; 9: 461–466.
19. Arkwright PD, Fujisawa C, Tanaka A, Matsuda H. Mycobacterium vaccae reduces scratching behavior but not the rash in NC mice with eczema: a randomized, blinded, placebo-controlled trial. J Invest Dermatol 2005; 125: 140–143.
20. Werner Y. The water content of the stratum corneum in patients with atopic dermatitis. Measurement with the Corneometer CM 420. Acta Derm Venereol 1986; 66: 281–284.
21. Jung K, Tanaka A, Fujita H, Matsuda A, Oida K, Karasawa K, et al. Peroxisome proliferator-activated receptor γ-mediated suppression of dendritic cell function prevents the onset of atopic dermatitis in NC/Tnd mice. J Allergy Clin Immunol 2011; 127: 420–429.
22. Matsumoto M, Itakura A, Tanaka A, Fujisawa C, Matsuda H. Inability of IL-12 to down-regulate IgE synthesis due to defective production of IFN-γ in atopic NC/Nga mice. J Immunol 2001; 167: 5955–5962.
23. Menon GK, Price LF, Bommannan B, Elias PM, Feingold KR. Selective obliteration of the epidermal calcium gradient leads to enhanced lamellar body secretion. J Invest Dermatol 1994; 102: 789–795.
24. Kubo A, Nagao K, Yokouchi M, Sasaki H, Amagai M. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. J Exp Med 2009; 206: 2937–2946.
25. Nagao K, Kobayashi T, Moro K, Ohyama M, Adachi T, Kitashima DY, et al. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. Nat. Immunol. 2012; 13: 744–752.
26. Togawa Y, Kambe N, Shimojo N, Nakano T, Sato Y, Mochizuki H, et al. Ultra-pure soft water improves skin barrier function in children with atopic dermatitis: A randomized, double-blind, placebo-controlled, crossover pilot study. J Dermatol Sci 2014; 76: 269–271.