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Effect of *São Pedro do Sul* thermal water on skin irritation

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Synopsis

Many mineral waters are known for centuries for treating dermatological diseases but there is little scientific evaluation of the effects of these waters in skin. A total of 17 healthy Caucasian volunteers, including men and women, were enrolled in this study. Two skin sites were marked on each volunteer forearm and irritated with sodium lauryl sulphate at 2% (w/v) kept under occlusion for 24 h with Finn chambers. Afterwards, purified water or *São Pedro do Sul* (SPS) thermal water were applied to the irritated skin sites, and kept under occlusion for 48 h also with Finn chambers. Transepidermal water loss (TEWL) was used as a measure of the skin barrier function to evaluate the potential anti-irritant effect of the thermal water on skin. Statistically significant differences in the mean TEWL variations were observed for the skin treated with SPS thermal water and with purified water ($P = 0.036$). The thermal water reduced the degree to which the skin barrier was disrupted compared with purified water alone in 82.4% of the volunteers. The SPS thermal water is anti-irritant and, therefore, can be helpful to relieve skin irritation and in cosmetic formulations to improve the tolerability of the products.

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Résumé

Effet de l'eau thermale de São Pedro do Sul sur l'irritation de peau

Beaucoup d'eaux thermales sont connues depuis des siècles pour traiter des maladies dermatologiques, mais il y a peu d'évaluation scientifique des effets de ces eaux sur la peau. Dix-sept volontaires sains de type Caucasiens, comprenant des hommes et des femmes, ont participé à cette étude. Deux zones ont été marquées sur chaque avant-bras et irritées avec du SLS à 2% (w/v) maintenu sous occlusion pendant 24 heures avec des Finn chambers. Ensuite, l'eau purifiée ou l'eau thermale de São Pedro do Sul (eau thermale SPS) a été appliquée sur les zones cutanées irritées et maintenues, également avec des Finn chambers, sous occlusion pendant 48 heures. La mesure de l'état de la barrière par évaporimétrie (TEWL) a été utilisé pour évaluer le possible effet anti-irritant de l'eau thermale sur la peau. Des différences statistiquement significatives des TEWL moyennes ont été observées entre la peau traitée avec l'eau thermale SPS et celle traitée avec l'eau purifiée ($P = 0.036$). L'eau thermale comparativement à l'eau purifiée a diminué de 82.4%l'impact de la destruction de la barrière cutanée des volontaires. l'eau thermale SPS est anti-irritante et peut donc être utile pour diminuer l'irritation cutanée et dans des formulations cosmétiques pour en améliorer la tolérance.

Introduction

Mineral waters are natural aqueous solutions formed under specific geological conditions [1, 2].

They have three fundamental characteristics: originate naturally from the earth as 'springs', are bacteriologically pure and have therapeutic potential. Many mineral waters are known for centuries for treating dermatological diseases, such as atopic dermatitis, psoriasis and contact dermatitis [1, 2]. However, the mechanisms by which these diseases are treated by spa therapy have not been yet fully elucidated.

On the basis of their chemical characteristics, waters may be classified as oligomineral waters (mineralization <200 mg L^{-1}), medium mineral waters (mineralization between 200 and 1000 mg L^{-1}) and mineral waters (mineralization above 1000 mg L^{-1}) [3].

The *São Pedro do Sul* (SPS) thermal water wells up at 68.7°C, is bacteriologically pure and is a medium mineral water, containing several mineral salts that make this water worldwide unique. The therapeutic characteristics of its water, the excellence of the thermal treatments and the natural beauty of the Lafões region, in the centre of Portugal, make this thermal centre the most visited in the country, with over 25 000 visitors in 2001 [4]. Table I shows the main components of SPS thermal water (bicarbonate, sodium and silica being the major components) and of other thermal waters.

Sodium lauryl sulphate (SLS) is a well known anionic surfactant that has been used extensively as a model irritant in the field of skin irritation testing [5]. In this study, the effect of the SPS thermal water on skin irritation induced by SLS was investigated.

Materials and methods

Subjects

A total of 17 healthy Caucasian volunteers, eight men and nine women, aged 21–42 years were enrolled in this study. All subjects received oral and written information about the modalities of the study, including the experimental protocol and the evaluation equipment to be used. Written informed consent was obtained. The principles stated in the Helsinki Declaration of 1975, and later revisions, were followed. Subjects were allowed to bathe daily, but washing the forearms with soap was prohibited.

Materials

Sodium lauryl sulphate 99% purity (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) was dissolved in purified water at 2% (w/v) concentration. SPS thermal water was obtained from its natural source 4 days before the beginning of the study.

Methods

The methods followed the guidelines on SLS exposure tests and the guidelines for transepidermal

Table I Chemical composition (mg L^{-1}) of some thermal waters [2, 26]

	SPS	Avène	La Roche-Posay	Vichy	Uriage
Silica (SiO_2)	65.5		30		
Total sulphur (in I_2 0.01n)	18.4				
Sodium (Na^+)	93	4.8	10	1860	2360
Potassium (K^+)	3.3	0.7	2.2	99.6	45.5
Calcium (Ca^{2+})	3	42.7	140	150.6	600
Ammonium (NH^{4+})	0.32				
Magnesium (Mg^{2+})	<0.03	21.2	4.9	12.3	125
Fluoride (F^-)	18.2				
Chloride (Cl^-)	27.7	5.4	25	357	3500
Bicarbonate (HCO^{3-})	126.9	226.7	396	4776.3	402
Carbonate (CO_3^{2-})	4.8				
Sulphate (SO_4^{2-})	9.7	14	30	Trace	2862
Nitrates (NO_3^-)	<0.12	1.4	Trace	Trace	<100
Silicate ($H_3SiO_4^-$)	12.4				
Selenium	<0.0012		0.06		
Dry residue	309	207	444	5120	11000

Total sulphur of the *São Pedro do Sul* (SPS) thermal water evaporates rapidly after water's extraction from its natural source.

water loss (TEWL) measurement from the Standardization Group of the European Society of Contact Dermatitis [5, 6].

Two test sites were marked in the forearm of each volunteer, at a minimum distance of 5 cm from the wrist. Forearm (left or right) and product applied to each site (SPS thermal water or purified water) were randomized using a table of random numbers.

On day 0, basal TEWL values were measured at both sites. 60 μL of the aqueous solution of 2% SLS was absorbed on filter papers (Ø 11 mm; Epitest Ltd Oy, Tuusula, Finland) applied in each site on the forearm. The filter papers were covered with large Finn Chambers (Ø 12 mm; Epitest Ltd Oy) fixed to the forearm using Scanpor® tape (Alpharma AS, Oslo, Norway) and kept under occlusion for 24 h.

On day 1, chambers were removed and the skin was rinsed with water and gently tapped dry. After 1 h with the test sites exposed to room temperature, TEWL was measured in each site. 60 μL of SPS thermal water or 60 μL of purified water was applied to each site, using the same procedure of the application of the aqueous solution of SLS, and kept under occlusion for 48 h.

On day 3, chambers were removed. After 1 h with the test sites exposed to room temperature, TEWL was measured in each site. TEWL values were assessed using a Tewameter TM 210 (Courage-Khazaka, Köln, Germany). The measurements were performed with the volunteer and the investigator in the sitting position. Noise in the room and talking during recordings were restricted. The values at equilibrium were registered 2 min after probe application to the skin and expressed as g m^{-2} h^{-1}. Room temperature was maintained at 21 ± 2°C and relative humidity at 60 ± 10%. Volunteers were allowed to rest at least 15 min before measurements in the laboratory area.

Transepidermal water loss differences between day 3 and day 1 were calculated for both sites. To compare the effect on TEWL paired Student *t*-tests were performed, after confirming the normal distribution of the data using the Shapiro-Wilk test. The level of significance was fixed at $\alpha = 0.05$. Statistical analysis was performed with SPSS 14.0 for Windows software (SPSS Inc., Chicago, IL, U.S.A.).

Results

Transepidermal water loss values obtained on day 1 at both sites showed no significant differences ($P > 0.05$). Therefore, the products were tested in equal damaged forearm skin sites.

Figure 1 shows the TEWL differences obtained with the SPS thermal water and with the purified water for each volunteer. In 82.4% of the cases these values were lower with the SPS thermal. Statistically significant differences in the mean TEWL differences were observed for the damaged skin treated with SPS thermal water and with purified water ($P = 0.036$) (Table II). Therefore, the thermal water had a statistically significant anti-irritant effect.

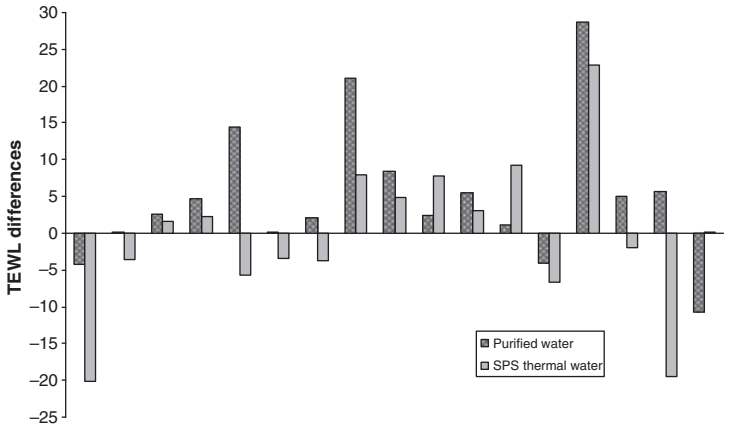

Figure 1 Transepidermal water loss differences between the values obtained before (day 1) and after (day 3) the *São Pedro do Sul* thermal water and the purified water application in the skin pre-irritated with sodium lauryl sulphate of 17 volunteers.

Table II Mean transepidermal water loss (TEWL) differences between day 3 and day 1

	São Pedro do Sul thermal water	Purified water
$TEWL_3 - TEWL_1$	−0.30 ± 10.24	4.89 ± 9.41

Results are expressed in g m^{-2} h^{-1} (mean ± standard deviation).

Discussion

Sodium lauryl sulphate exposure tests are commonly used to access the efficacy of different moisturizers or barrier creams in preventing or healing skin irritation [3]. SLS acute exposition has effects on the stratum corneum, on viable epidermis and on dermis leading to erythema, sometimes associated with infiltration and with superficial erosion of the epithelium. During healing of acute reactions, scaling and fissuring will take over.

When the skin surface is disrupted, its ability to avoid water loss is diminished [7]. Therefore, TEWL is a highly sensitive and precise measure of SLS irritant effects on skin and, consequently, of the skin barrier function [5–7].

In this study, as expected, 24 h exposition of forearm skin to an aqueous solution of SLS 2% under occlusion led to an increase of the TEWL values. Increases in the TEWL values until 2 or 3 days after irritation induced by SLS had been seen in many studies [3]. Katsarou *et al.*[8], in a very similar study, found TEWL increases until day 2 after SLS exposition. In our study, many of the TEWL differences obtained are positive values. Therefore, in many cases there was an increase in the TEWL until 3 days after SLS exposure. Despite this fact, the TEWL differences were statistically different, being lower with the thermal water in 82.4% of the cases. Therefore, the SPS thermal water had a statistically significant anti-irritant effect, increasing the rate of recovery of the skin barrier function.

Other waters had also shown to influence the recovery of the skin barrier function. Leopoldine spa water had an inhibitory effect on irritation caused by SLS [9]. Sea water obtained from the Pacific Ocean at San Francisco and 500 mmol L^{-1} NaCl and 10 mmol L^{-1} KCl solutions improved skin barrier after exposition to SLS when compared with purified water [10]. An *in vivo* study carried out in skin of hairless mice analysed the effects of four different magnesium salts and a mixture of magnesium and calcium salts on the cutaneous barrier recovery rate after barrier disruption [11]. All the magnesium salts (magnesium chloride, magnesium lactate and magnesium sulphate), except magnesium bis(dihydrogen phosphate), accelerated barrier repair. The mixture of magnesium and calcium salts, when the calcium to magnesium ratio was lower than 1, also accelerated barrier repair. Bock *et al.* [12] found that a CO_2-enriched water improved the barrier recovery of detergent-damaged skin. These findings suggest that these waters may have common chemical properties that influence the recovery of the skin barrier function.

However, the physiological mechanisms that could explain these effects are still poorly understood. It is well recognized that there is a calcium gradient in normal skin with high concentrations of calcium in the outermost layers of the epidermis [13–15]. Disruption of the skin barrier results in a decrease of calcium levels in the outer epidermis, which then signals the outer stratum granulosum cells to secrete their pool of lamellar bodies. If the calcium levels in the outer epidermis are prevented from decreasing after barrier disruption, lamellar body secretion does not occur and the barrier repair process is not initiated [16]. Similar behaviours were observed with gradients of potassium and magnesium after barrier disruption [15, 16]. Bock *et al.* [12] suggested the formation of calcium bicarbonate and potassium bicarbonate to explain the improvement of the barrier recovery caused by a CO_2-enriched water. The formation of the salts would cause a decrease of calcium and potassium in the outer epidermis that then signals for skin recovery. Based in this theory, it can be hypothesized that the anionic ions of thermal waters are mainly responsible for the anti-irritant effect.

On the other hand, it's well known that moisture plays an important role on skin barrier function [17]. Several studies showed that moisturizing creams can improve barrier repair after damage [18–20]. Generally, this effect has been related with the replacement of stratum corneum lipids, reducing TEWL by the tissue. However, the skin has also the ability to selectively retain water by the natural moisturizing factor (NMF) [10]. Chloride, sodium, potassium, ammonia, calcium, magnesium and phosphate are components of NMF. These minerals are capable of restoring moisture as a result of their

hygroscopic characteristics. Biologically, this activity allows the outermost layers of the stratum corneum to remain hydrated despite the desiccating action of the environment. Therefore, the ability of some specific waters, including the thermal water tested in this study, to accelerate the recovery of the skin barrier function, may also be related with the functionality of the NMF.

Some waters had proven to influence the immune system, to have anti-inflammatory activity or to be anti-oxidant [1]. Two *in vitro* studies showed that Avène thermal water inhibit mast cell activation [21, 22]. This activity may be in part responsible for its anti-inflammatory properties. It was found later, that calcium and bicarbonate ions mediate the inhibition of mast cell histamine release by this water [23]. Other studies demonstrated that Avène spa water has also an inhibitory effect on vasoactive intestinal peptide-induced inflammation in skin that can also explain its properties. The Uriage thermal water induced the apoptosis of human eosinophils in an *in vitro* study [24]. This effect was related to the calcium content of this water. The topical application of La Léchère thermal water activated the cutaneous microcirculation *in vivo* [25]. La Roche-Posay water demonstrated to have an anti-oxidant effect, which was related to its component selenium [1].

These studies suggest that thermal waters may have different effects that contribute to the anti-inflammatory activity in the skin and to improve skin barrier after damage. However, the mechanisms involved and its relation with the thermal waters composition are still poorly understood.

In summary, the *São Pedro do Sul* thermal water promoted the barrier recovery of the skin after irritation induced by SLS. These results emphasize that this thermal water has an anti-irritant effect and, therefore, it may be helpful to relieve skin irritation. As some cosmetic products can induce skin irritation, this thermal water may also be helpful to enhance the tolerability of these formulations.

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Conflict of interest

The authors have no conflict of interest.

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