

## Analgesic effect of mineral cream containing natural spa minerals for use on the skin

Takahiro HIRABAYASHI<sup>1\*</sup>, Michio YAMASHITA<sup>1, 2\*</sup>, Nobuhiro WADA<sup>1</sup>, Fumiko TAKENOYA<sup>2</sup>, Hiroko IKEDA<sup>3</sup>, Junzo KAMEI<sup>3</sup>, Tomoo RYUSHI<sup>4</sup>, Noriyuki YAMAMOTO<sup>5</sup>, and Seiji SHIODA<sup>1</sup>

<sup>1</sup>Global Research Center for Innovative Life Science, Hoshi University, Tokyo, Japan; <sup>2</sup>Department of Exercise and Sports Physiology, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Tokyo, Japan; <sup>3</sup>Department of Pathophysiology and Therapeutics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Tokyo, Japan; <sup>4</sup>Faculty of Sports and Health Science, Department of Sports Science, Daito Bunka University, Saitama, Japan; and <sup>5</sup>Department of Health Sciences, Japanese Red Cross Hokkaido College of Nursing, Kitami, Japan

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### ABSTRACT

Previous studies have shown that dissolved substances in some natural hot springs have analgesic/anti-nociceptive and anti-inflammatory actions. However, the mechanisms underlying how such dissolved substances exert these actions are not fully understood. In the present study on mice, we examined the analgesic/anti-nociceptive and anti-inflammatory properties of a mineral cream containing natural hot spring ingredients. The anti-nociceptive effects of the mineral cream were assessed by using the von Frey test. Application of the mineral cream to the hind paw of mice produced a significant anti-nociceptive effect compared to control. The anti-nociceptive effects of the mineral cream were also assessed following the injection of complete Freund's adjuvant (CFA) into the hind paws of mice after pre-treatment for one or four weeks with the mineral cream. Histological experiments with light microscopy showed that the mineral cream did not reduce inflammation caused by the CFA treatment. In addition, the mineral cream did not inhibit oxidative stress as evidenced by increased levels of oxidative metabolites (d-ROMs) and biological anti-oxidant potential (BAP). These results suggest that the mineral cream does not exert a protective effect against inflammation, and that the constituents of the mineral cream may produce their anti-nociceptive effects transdermally via different mechanisms including the nervous system.

Balneotherapy and/or spa therapy are commonly used worldwide to treat rheumatic conditions (12, 20) such as lower back pain (19), knee osteoarthritis (11), hand osteoarthritis (10) and fibromyalgia (3, 27). Balneotherapy has also been shown to improve chronic shoulder pain and joint function (37), while spa therapy has been described to improve pain, functionality, and quality of life in patients with

chronic shoulder pain (4). Spa therapy can be roughly classified into three mechanistic actions: a physical action, a normalization effect on the autonomic nervous system, and a chemical action (4).

In Japan, hot spring therapy has been used for centuries and its medicinal effects have transcended into modern medicine. Spa therapy (hot spring therapy) treats physical conditions by way of bathing, drinking, inhaling, treating wounds, conditions, etc., in hot spring water. In addition, some hot springs claim beautifying effects on the skin. The chemical action of hot springs is such that components contained in spring water, such as carbon dioxide, salt,

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Address correspondence to: Dr. Seiji Shioda, Global Research Center for Innovative Life Science, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

Tel: +81-3-5498-5853, Fax: +81-3-5498-5853

E-mail: shioda@Hoshi.ac.jp

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\*These authors contributed equally to this work.

gypsum, aluminum, sulfur, and trace levels of radioactivity, act on the body in a medically beneficial way. However, some aspects of hot springs impact negatively on the human organism, meaning that use of the springs is contraindicated in some patients with diseases or medical conditions such as atopy (17, 23, 35), hemorrhoids, gastrointestinal diseases, rheumatism (6, 9, 36), lower back pain (2, 7, 22), neuralgia, hypertension (25), burns, bone fractures, psychiatric disorders and the like.

Some spa therapies and balneotherapies have been reported to inhibit inflammatory skin disorders by suppressing oxidative stress (8). An analgesic effect of natural hot spring ingredients has been shown following application of these ingredients to the skin of animals (25). Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant system (antioxidant substances and antioxidant enzymes). Oxidative stress caused by reactive oxygen species is considered a major mediator of tissue injuries, and excess amounts of the ROS have been recorded immediately after brain ischemia-reperfusion prior to a rise in neuronal cell death (28, 29). When the state of high oxidative stress is long-lasting, DNA, proteins, lipids including cell membrane, and carbohydrates in the body undergo oxidative damage, and nitric oxide synthesis may induce ongoing pathological diseases (18); this is commonly seen in diseases such as arteriosclerosis, blood coagulation (30). For example, in diabetes, it is known that oxidized sugar and protein bind to form glycosylated proteins. Also, in blood vessels affected by arteriosclerosis, oxidized lipid accumulates in the lumen of the blood vessels, causing them to narrow and to impede blood flow (40). Several other studies with experimental animals have shown that thermal stress activates ROS production (16). It is considered important to reduce ROS damage and to increase antioxidant activity which decreases as age progresses. In this way, oxidized proteins accumulate in the brain, particularly in diseases such as Alzheimer's disease and Parkinson's disease (34, 26, 32), which are more common in elderly people.

In the present study we examined the anti-nociceptive and anti-inflammatory properties of a mineral cream containing spa ingredients, which has been shown to have fatigue-reducing, anti-nociceptive and anti-inflammatory properties in athletes and other populations, but whose underlying scientific mechanisms have yet to be elucidated. To this end, we used mice to investigate the influence of the mineral cream on pain sensation assessed by the von Frey test.

Furthermore, we studied whether the mineral cream inhibits inflammation induced by complete Freund's adjuvant (CFA). Pathophysiological changes were assessed by light microscopy. In addition to studying mechanisms underlying the anti-nociceptive effects of the mineral cream, we measured oxidative metabolites (d-ROMs) and the biological anti-oxidant potential (BAP) to assess if the mineral cream could reduce reactive oxidative stress induced by the CFA test.

## MATERIALS AND METHODS

*Spa mineral cream.* Skin cream containing natural spa minerals (mineral cream) was provided by SHOKKEN Co. Ltd. (Tokyo, Japan). This mineral cream contains dissolved ingredients from the Hakodate spa (Hokkaido, Japan). The chemical composition and concentration of constituents in the spa water are listed in Table 1.

*Animals and treatment.* Male ICR mice (7-weeks-old) weighing 30–35 g were obtained from Tokyo Laboratory Animals Science Inc. (Tokyo, Japan). The animals were placed into experimental groups in cages and maintained in an airconditioned room (temperature:  $24 \pm 1^\circ\text{C}$ ) with a 12-h light-dark cycle (lights on at 8 : 00, off at 20 : 00). All animals had *ad libitum* access to standard pellet feed and water. The animals were habituated to the laboratory environment for 48 h prior to the experiments. After conditioning the mice, they were divided into three groups consisting of a control group (no treatment with cream, 5 animals), a vehicle group (treated with the cream base (vehicle) only, 5 animals), and the main experimental group (cream application containing spa minerals, 5 animals). Cream or vehicle was applied once per day (about 150 mg) to the soles of the paws and hindlimbs (a total of 4 places) of mice from the experimental and vehicle-treated groups for 1 week or 4 weeks. All animal experiments were approved by the Animal Care Committee at Hoshi University (Tokyo, Japan) and carried out in accordance with the guide for the care and use of laboratory animals of Hoshi University, Tokyo, which is accredited by the Ministry of Education Culture Sports Science and Technology of Japan.

*von Frey filament test.* Mechanical sensitivity was determined by probing the plantar surface of the left hind paw (von Frey test) with a plastic filament of a dynamic plantar aesthesiometer (Ugo Basile, Comerio, Italy). Force was applied to the hind paw at a

**Table 1** Mineral composition of spa cream

Cation		mg/Kg	Anion		mg/Kg
Hydrogen	H <sup>+</sup>	6.8	Fluorine	F <sup>-</sup>	0.5
Sodium	Na <sup>+</sup>	70.8	Chlorine	Cl <sup>-</sup>	187.5
Potassium	K <sup>+</sup>	9.2	Hydrogen sulfide	HS <sup>-</sup>	–
Ammonium	NH <sub>4</sub> <sup>+</sup>	3.5	Dithionous acid	S <sub>2</sub> O <sub>4</sub> <sup>2-</sup>	–
Magnesium	Mg <sup>+</sup>	31	Hydrogen sulfate	HSO <sub>4</sub> <sup>-</sup>	617.8
Calcium	Ca <sup>+</sup>	276.7	Sulfate	SO <sub>4</sub> <sup>-</sup>	2908
Aluminum	Al <sup>3+</sup>	325.7	Dihydrogen phosphate	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	1.7
Manganese	Mn <sup>+</sup>	1.6			
Ferrous	Fe <sup>2+</sup>	125.4			
Ferric	Fe <sup>3+</sup>	48.5			
Zinc	Zn <sup>+</sup>	0.2			

rate of 0.25 g/s; the final force when paw withdrawal was observed was measured automatically (mechanical threshold). A maximal cut-off of 5 g (force) or 20 s (time) was used to prevent tissue damage. The mechanical threshold was determined as the average of three measurements per mouse.

*Adjuvant-induced inflammation.* We used a unilateral adjuvant-induced inflammation model as previously described (5). In this model, the left footpads of mice were injected subcutaneously with 20  $\mu$ L of complete Freund's adjuvant (CFA: mycobacterium tuberculosis; Sigma-Aldrich Co., LLC, undiluted) with a 27G needle into the plantar surface. Twenty-four hours after the CFA injection, the vehicle or mineral cream was applied to the skin of the left hind paw of each mouse (control group, vehicle group, mineral cream group) every day for 4 days. The time course of the inflammatory-related response was assessed by measuring two response parameters (paw diameter, von Frey test) as described above. The test was repeated three times at intervals of 5 min, and the average value in grams and time was calculated. After completion of the von Frey test, the thickness of the foot was measured as an index of edema. Measurements were made with calipers before the experiments, and at various times after treatment.

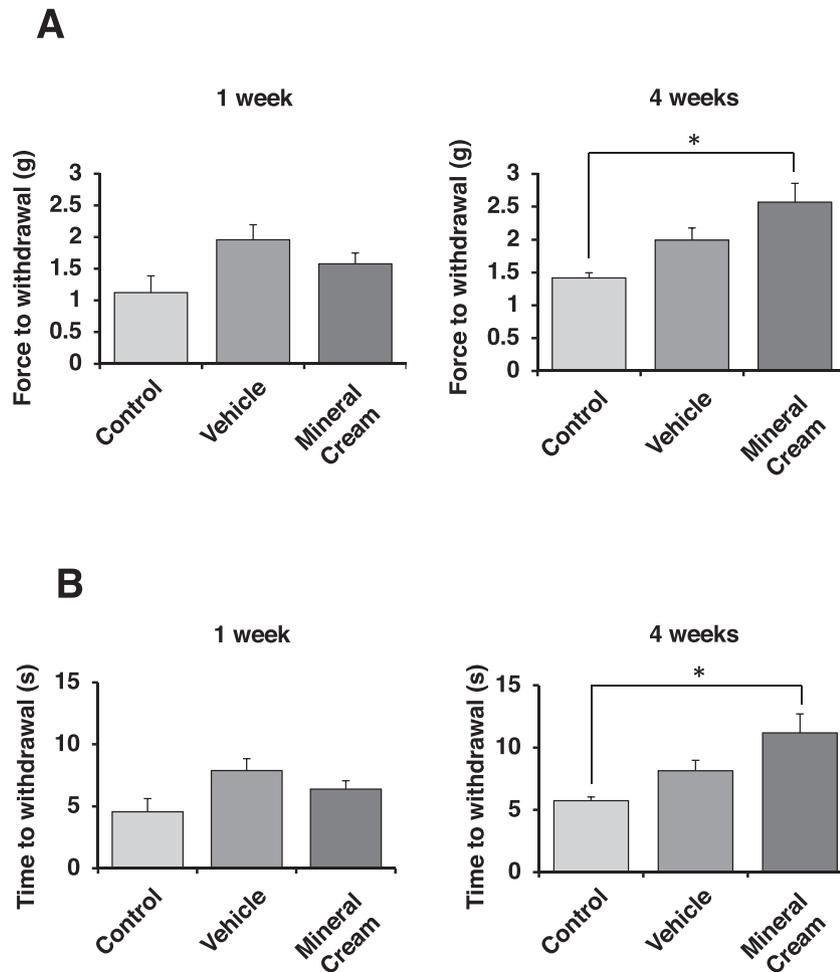
*Determinations of oxidative stress (d-ROMs).* In the d-ROMs test, we quantified oxidative stress by capturing the metabolite ROOH rather than directly measuring active oxygen and free radicals. A commercial kit was used that involved colorimetric determination of ROS (d-ROMs) using Free Radical Electron Evaluator (FREE; Health & Diagnostics, Naples, Italy) as per the manufacturer's instructions. Briefly, the anti-oxidant capacity of the spring mineral-containing cream was measured from blood samples collected

immediately at the end of the 1- and 4-week mineral cream application periods. Heparinised blood was applied to a centrifugal separator (6000 rpm, 5 min), and plasma was collected. Next, the plasma was analyzed with a free radical analyzer (WISMERLL, Tokyo), and the values of oxidative stress (d-ROM) and BAP were measured.

*Assay for anti-oxidative potential.* Anti-oxidative potential was measured with a commercial kit (Biological anti-oxidant potential (BAP) test) using FREE according to the manufacturer's instructions with minor modifications. Briefly, plasma aliquots were mixed with reactive solutions and the absorbance determined at 510 nm immediately prior to initiation of the reaction. The mixture was then incubated for 5 min at 37°C, and the post-reaction absorbance of the mixture was measured as previously described (29).

*Histopathological analysis.* Mouse metatarsal foot pads were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer overnight at 4°C and then exposed to hydrochloric acid and formic acid decalcification solution for one week at room temperature. The tissue samples were dehydrated through a graded ethanol series and then embedded in paraffin. Tissue sections (thickness 6–7  $\mu$ m) were prepared with a microtome and stained by hematoxylin and eosin (H&E) for subsequent study by light microscopy.

*Statistical analysis.* The results were expressed as mean  $\pm$  standard error of the mean (S. E. M.). Statistical significances within groups were analyzed by one- and two-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparisons test. P-values less than 0.05 ( $P < 0.05$ ) were considered significant.



**Fig. 1** Anti-nociceptive effect of the mineral cream following short-term and long-term application. Effect of the mineral cream applied for 1 week (left panel) and 4 weeks (right panel) as measured by the von Frey test. The vertical axis represents the paw withdrawal threshold measured in response to applied force using von Frey filaments (grams, A) and in terms of time (seconds, B). Values are expressed as the mean  $\pm$  SEM. \* $P < 0.05$  compared to control group.

## RESULTS

### *Anti-nociceptive effect of mineral cream assessed using the von Frey filament test*

Pain avoidance one week or four weeks after daily application of the mineral cream was assessed using the von Frey filament test. For the one-week application period, the vehicle group showed a tendency towards an increased time and higher pressure to produce an avoidance reaction, but no significant difference was found between the three groups. However, for the 4-week application period, significantly increased time and pressure values prior to eliciting a pain avoidance response were recorded for the experimental group (cream containing spa minerals) compared to the control group (Fig. 1A, B). From these results, it was evident that long-term applica-

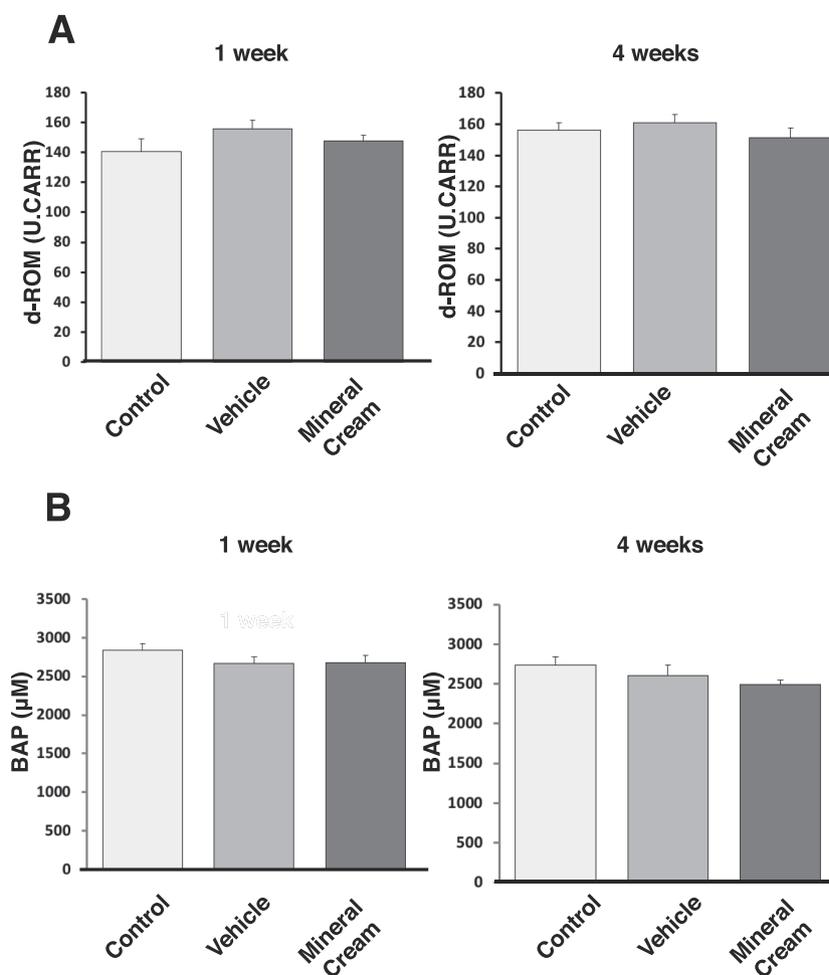
tion of the mineral cream was effective in reducing pain in response to stimulation.

### *Effects of mineral cream on oxidative stress*

The influence of mineral cream application on tissue antioxidant properties (d-ROM and BAP values) were studied. No differences in d-ROM (Fig. 2A) or BAP (Fig. 2B) values were found between any of the three groups following 1- and 4-week applications of cream. These results suggest that the mineral spring cream had little or no effect on antioxidant properties in treated hindpaw tissue.

### *Adjuvant-induced inflammation model*

The anti-nociceptive effects of the mineral cream were measured by the von Frey test 24 h following the injection of CFA into the left hindpaw of mice.



**Fig. 2** Effect of the mineral cream on oxidative stress following short-term and long-term application. Effect of the mineral cream applied for 1 week (left panel) and 4 weeks (right panel) on oxidative stress. (A) d-ROM, (B) BAP.

Twenty-four hours after the CFA injection, vehicle or mineral cream was applied to the skin of the left hindpaw of each mouse (control group, oil group) and the withdrawal threshold against mechanical stimulation by a von Frey filament applied to the plantar surface of each hind paw was measured. This test was repeated each day for four days. The mineral cream potently and significantly increased the reaction threshold in time (Fig. 3A) and force (Fig. 3B) by at least two days compared with the control group, suggesting that the mineral cream has analgesic effects in response to CFA-induced inflammation.

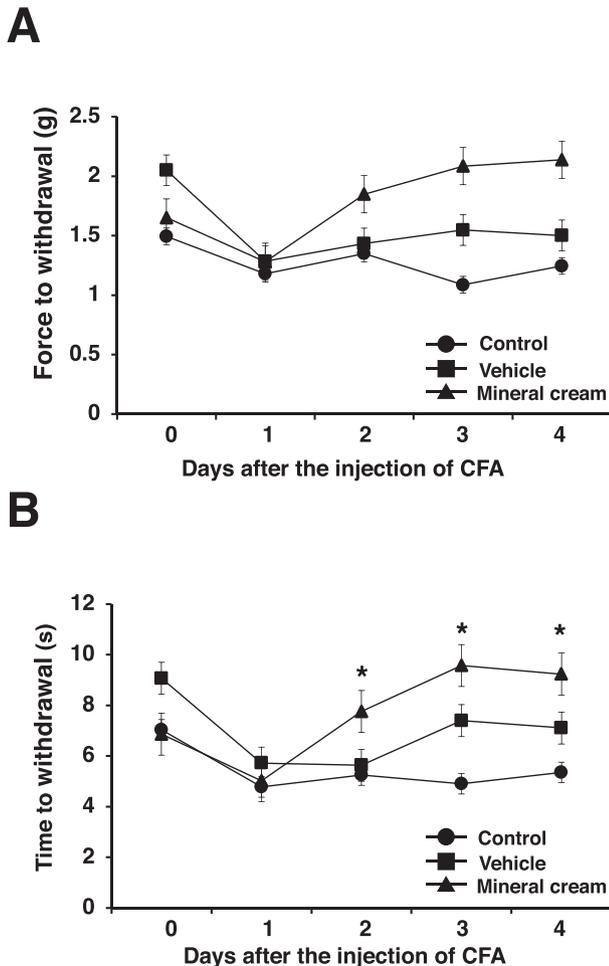
#### *Effects of mineral cream on CFA-induced swelling*

The injection of CFA into the left hindpaw of mice induced swelling, with a peak in paw edema seen 24 h after the CFA injection. No significant differences were observed between three groups (Fig. 4).

In addition, anatomical analysis by H&E staining demonstrated that no significant differences among groups were evident by day 4 in terms of the number or distribution of inflammatory cells in the hindpaws of mice induced by CFA (Fig. 5, 0d). The mineral cream thus had a little or no anti-inflammatory effect on CFA-induced inflammation within the first four days (Fig. 5, 4d).

#### DISCUSSION

In the present study, the mineral cream that was tested on the hindpaws of mice produced anti-nociceptive but not anti-inflammatory effects. The results showed that a long-term application for four weeks was necessary to achieve significantly increased time and pressure values to produce an avoidance reaction in the von Frey filament test. It is thus expected that people using this cream would need to undergo dai-



**Fig. 3** Anti-nociceptive effect of the mineral cream on CFA-induced allodynia. Effect of the mineral cream as measured by the von Frey test 24 h after the injection of CFA and on each day for a further three days. The vertical axis represents the paw withdrawal threshold measured in response to applied force using von Frey filaments (grams, A) and in terms of time (seconds, B). Values are expressed as the mean  $\pm$  SEM. \* $P < 0.05$  compared to control group.

ly treatment for at least one month in order to obtain adequate pain relief effects.

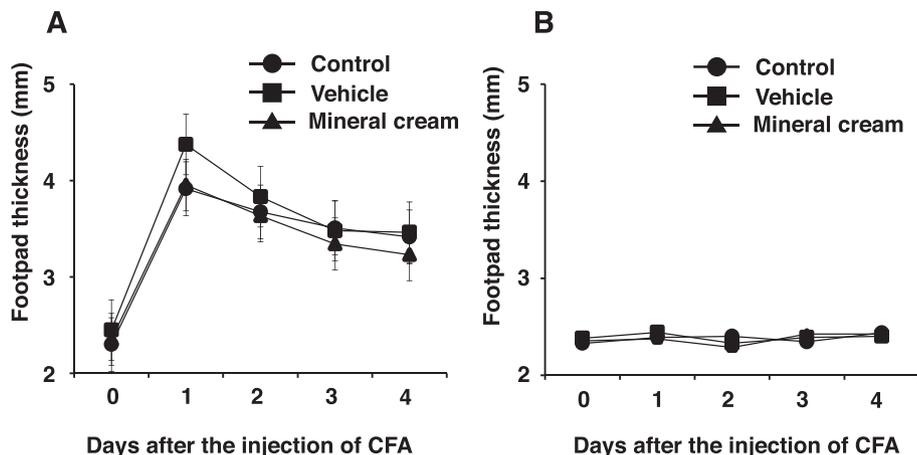
As described in Table 1, the mineral cream contained elevated levels of calcium, aluminum, ferrous cations, hydrogen sulfate and sulfate anions. Recently spa therapy together with supervised self-mobilization was shown to improve pain, joint function and quality of life in patients with chronic shoulder pain (13, 14). Rühle *et al.* (2018) reported that radon/carbon dioxide spa treatment efficiently reduces pain (31), which is consistent with previous reports of pain inhibition in rheumatoid arthritis by spa waters containing sulfate (1, 2), magnesium (33), carbon dioxide (15) and radon (13). While we have

shown that the mineral cream has anti-nociceptive effects, it is not yet known which minerals or ions in the mineral cream were responsible for this. Future work will concentrate on clarifying which components of the cream have analgesic properties.

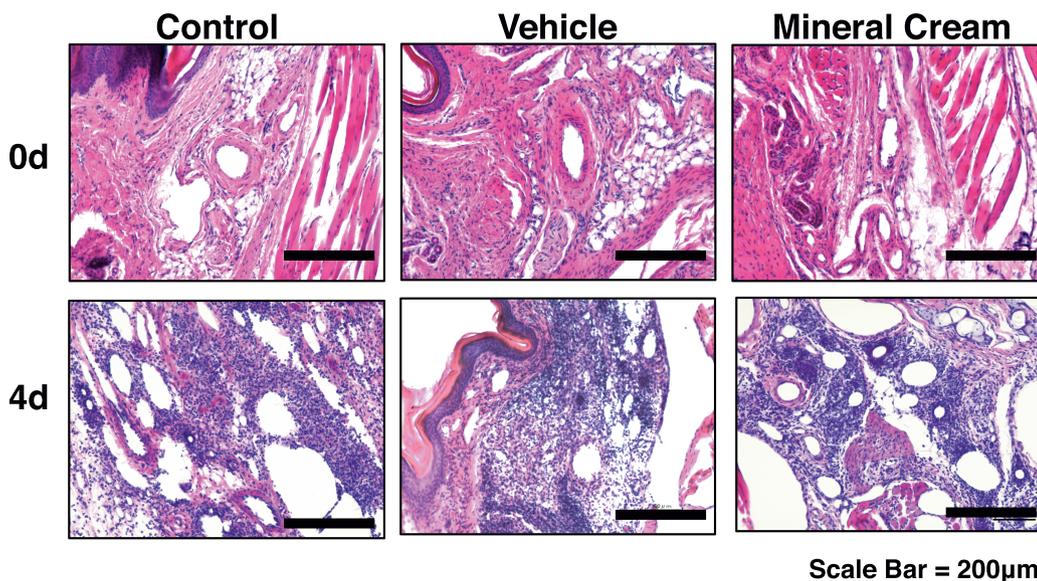
As to the inflammation induced by the injection of CFA into the hindpaw, the mineral cream has analgesic effects as shown in Fig. 3. However, the mineral cream has no apparent inhibitory effects on swelling and the number or distribution of inflammatory cells in the hindpaws as shown in Fig. 4 and Fig. 5. It may be possible that different findings will be obtained if the inflammatory reaction conditions (CFA test) were changed or if the number of days of observation was increased further. Furthermore, it is also possible that mineral cream has a local anaesthetic effects. As for this possibility, it is necessary to study the physiological effect of the mineral cream on pain sensory nerve by use of different experimental methods such as electrophysiological analysis.

We studied the anti-oxidant and anti-inflammatory effects of the mineral cream by measuring d-ROM and BAP values, but no significant effects were detected. The d-ROM test is a numerical value obtained by analyzing hydroperoxide of metabolites of active oxygen/free radicals in blood. Taken together, our data suggest that the anti-nociceptive effect of mineral cream was not due to an increased antioxidant capacity in the treated hindpaw tissues. Recently, it was reported that radon inhalation increases superoxide dismutase activity in the central and peripheral organs of mice, suggesting the activation of anti-oxidative functions (21), which may provide symptom relief by reducing ROS levels.

Although an anti-nociceptive effect was obtained here by applying the mineral cream to the skin, how can the mechanism of action underlying the analgesic effect be considered? In aromatherapy treatment, the systemic and transdermal administration of ginger essential oil to animals also induces anti-nociceptive effects (5, 24). We previously demonstrated a possible involvement of spinal nerve activity suppression induced by essential oils to regulate the transmission of pain stimuli in rats via anti-inflammatory and analgesic effects (5). Although the analgesic effects of the mineral cream used in this study are evident, the underlying mechanism of action clearly needs to be elucidated. The properties of the cream may nevertheless be highly valuable for use in palliative care. While further research is required, our findings will add to the body of knowledge of the importance of mineral cream containing natural spa ingredients as



**Fig. 4** Effects of mineral cream on CFA-induced swelling. Effects of the mineral cream on CFA-induced swelling were evaluated by measuring footpad thickness 24 h after after CFA injection. (A) CFA injected (left) footpad; (B) Non-injected (right) footpad.



**Fig. 5** Effects of mineral cream on CFA-induced inflammation by histological analysis. H&E staining of footpad 24 h after CFA injection.

an adjunct to standard medications in the treatment of patients and people with soft tissue injuries.

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