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Eye and skin irritation tests to assess the safety of Jeju saline groundwater

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Abstract Saline groundwater was obtained from a 100-m deep basalt layer into which seawater had infiltrated. This groundwater is known to contain various minerals. Like deep seawater, saline groundwater is expected to have various applications due to the presence of biologically beneficial minerals. In Korea, saline groundwater is mainly obtained from the coastal area of Jeju. Before applying saline groundwater to various products, however, its biological safety needs to be examined. In the present study, skin and eye irritation tests were performed to assess the safety of saline groundwater according to the guidelines of the Korea Food and Drug Administration. When compared to control, Jeju saline groundwater showed no level of eye and skin irritation. These results suggest that Jeju saline groundwater induces no irritation, and is therefore sufficiently safe to be applied to the eye and skin of people.

Keywords Eye irritation test · Magnesium · Mineral · Saline groundwater · Skin irritation test

Introduction

Inorganic materials, such as minerals, are beneficial to human health, and some are essential for biological processes, such as nerve transmission and maintenance of osmosis. However, people do not uptake inorganic materials adequately (Hwang et al. 2016). The importance of many kinds of minerals has been reported consistently. For example, magnesium deficiency is associated with hypertension, atherosclerosis, and diabetes mellitus (Ma et al. 1995). Thus, people must ingest adequate amounts of minerals for their health and survival.

Saline groundwater in coastal areas, especially Jeju saline groundwater, is mainly the result of saline water intrusion into lava rock and their subsequent chemical reaction (Kim et al. 2003). Because saline groundwater is a mixture of seawater and fresh water, it contains many inorganic ions, including sodium (Na^+), chloride (Cl^-), and magnesium (Mg^{2+}), just like seawater. Thus, saline groundwater could be an excellent source of minerals, and could be used to as raw materials for drink, food, and cosmetics.

The physiological effects of saline groundwater are not well studied. However, deep seawater, which shares many properties with saline groundwater, has been animatedly studied by many researchers. Deep seawater was shown to lower hypertension and reduce serum cholesterol and triacylglycerol levels (Sheu et al. 2013). The fact that deep seawater and saline groundwater have similar compositions suggests that they will produce similar physiological effects; however, saline groundwater may not be as safe as deep seawater. Thus, before its application to various fields, the safety of saline groundwater should be examined. In the present study, we performed eye and skin irritation tests to assess the safety of saline groundwater from Jeju Island.

Materials and Methods

Saline groundwater

Generally, the total dissolved solid (TDS) of saline water is between 10,000 and 100,000 mg/L (Freeze and Cherry 1979). Saline groundwater was collected from Jeju, Republic of Korea, and its average TDS content was more than 35,000 mg/L. The

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main components of this saline groundwater were 57.7% chloride ion (Cl^-), 28.2% sodium ion (Na^+), 8.2% sulfate ion (SO_4^{2-}), 3.4% magnesium ion (Mg^{2+}), 1.2% potassium ion (K^+), and 1.1% calcium ion (Ca^{2+}). Jeju saline groundwater was prepared and provided by Jeju TechnoPark, Republic of Korea.

Animals

New Zealand White rabbits (KOATECH, Pyeongtaek, Korea) were used as the animal model to test whether Jeju saline groundwater induces eye and skin irritation. The animals were maintained at 20±2 °C, and 50±5% humidity, with a 12-h light-dark cycle. They had free access to food (Cargill Agri Purina, Seongnam, Korea) and tap water. Before starting the irritation tests, the body weights of all rabbits needed to be more than 2.0 kg. All the procedures in this research were approved by the Korea University Institutional Animal Care and Use Committee (KOREA-2016-0067).

Eye irritation test

Eleven rabbits were used to observe irritation and defects in their eyes. Five percent (w/v) sodium fluorescein (Sigma-Aldrich, St. Louis, MO, USA) was applied to the eyes, which were examined under blue light at 495 nm. The presence of corneal lesions, irritations, or defects results in the appearance of green light, emitted at 520 nm. After verifying the absence of corneal lesions, the residual sodium fluorescein was washed out using physiological saline solution (0.75% w/v NaCl). The eye irritation test was conducted 24 h later, according to the guidelines of the Ministry of Food and Drug Safety (Chungbuk, Korea). Ten rabbits received 0.1 mL of saline groundwater in the left eye, while the other rabbit received 0.1 mL of saline groundwater in its right eye due to an observed defect in the left eye. The opposite eye was not treated and was used as a negative control in each rabbit. Rabbits were divided in two groups, unwashed and washed. The unwashed group consisted of seven rabbits and the washed group consisted of four rabbits. The eyes of the latter group were washed with physiological saline after treatment with Jeju saline groundwater. We observed the eyes six times after treatment, on days 1, 2, 3, 4, 6, and 7. The eye irritation score was estimated five times, using scoring charts of the guideline, on days 1, 2, 3, 4, and 7. Photos were taken four times, on the day of treatment, and on days 2, 4, and 6.

Skin irritation test

For the skin irritation test, seven rabbits were anesthetized to remove the hair on an area of their backs. Four squares, of dimension 2.5×2.5 cm^2, were marked in the glabrous area. Among the squares, the two squares on the left were scratched with a syringe needle; the two squares on the right side were not scratched. On the upper two squares, 0.5 mL of Jeju saline groundwater was applied. The two treated upper squares were covered with sterile gauze of the same size as the square, and nonirritant tape was used to fix the gauze in place. After 24 and 72 h, the degree of skin irritation was scored according to scoring charts in the guideline of the Ministry of Food and Drug Safety. Photographs were recorded.

Results and Discussion

In the eye irritation test, Jeju saline groundwater did not cause any irritation or abnormality in the treated eyes. The scoring chart of

Table 1 Eye irritation evaluation after treatment with Jeju saline groundwater*

Day	Rabbit number	Unwashed							Washed			
		1	2	3	4	5	6	7	8	9	10	11
Day 1	Cornea	0	0	0	0	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0
Day 2	Cornea	0	0	0	0	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0
Day 3	Cornea	0	0	0	0	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0
Day 4	Cornea	0	0	0	0	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0
Day 7	Cornea	0	0	0	0	0	0	0	0	0	0	0
	Iris	0	0	0	0	0	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0

*All eye irritation scores were 0. The scores were obtained by accessing corneal opacity, reactivity of iris, conjunctival edema, and ocular discharge. None were observed

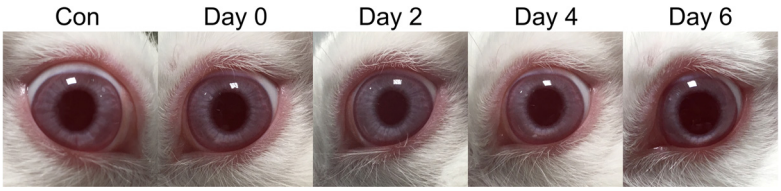

Fig. 1 Representative photos of the eye irritation test. The untreated eye was used as control (Con). The cornea, iris, and conjunctiva were observed on days 1, 2, 3, 4, and 7. Photos were taken on days 0 (before starting eye irritation), 2, 4, and 6. In this study, no differences of cornea, iris and conjunctiva between day 0 and day 7 were observed. These photos are of one representative rabbit

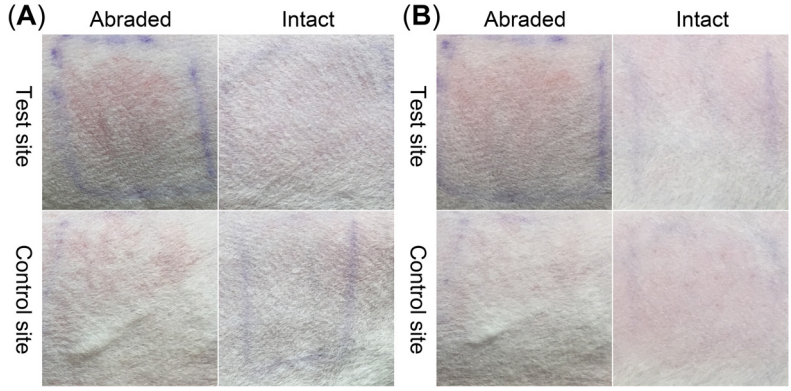

Fig. 2 Representative photos of the skin irritation test. The photos were taken 24 h (A) and 72 h (B) after treatment. Abraded sites were scratched with a syringe needle. Test sites received Jeju saline groundwater. Purple lines were drawn with a non-irritating pen. No differences in score were observed between the test and control site regardless of the skin being abraded, as shown in Table 2. These photos are of one representative rabbit

the guideline included checklist items of corneal opacity, reactivity of iris, chemosis, and discharge of eyes. When we scored five times for 7 days, no disorder was observed in the eyes of the rabbits (Table 1). Figure 1 shows one example of the eye irritation test. The different pupil sizes in the photos of Fig. 1 were due to varying light levels at the time when the photos were taken. The control photo was of the eye opposite to the treated eye. The photo on day 0 for the tested eye was taken before treatment with Jeju saline groundwater. The other photos on days 2, 4, and 6 were taken after treatment. Thus, the results of Table 1 and Fig. 1 clearly show that there were no differences between control and any day after treatment, regarding abnormality of the cornea, iris, and conjunctiva.

In the skin irritation test, the degree of irritation was assessed by scoring charts in the guideline. The items of skin irritation on the scoring charts consisted of erythema, eschar, and edema. The degree of irritation of the skin was checked twice over 3 days, once at 24 h and once at 72 h. Table 2 shows that no differences were observed between the control and test sites in both intact and abraded sites with respect to erythema, eschar, and edema. Fig. 2 shows the photos taken for the intact and abraded sites of control and test sites, after 24 h (A) and 72 h (B). In Fig. 2, while the control sites did not receive Jeju saline groundwater, the test sites were treated with Jeju saline groundwater. Regardless of groundwater treatment, the abraded sites were more reddened than the intact sites due to scratches with the syringe needle. As can be seen in Fig. 2, eschar, edema, and erythema were not observed on the back of the rabbits.

Although seawater contains large amounts of minerals known to play important roles in human physiology, it may not be easy to use it directly in commercial products due to possible contamination. Deep seawater and saline groundwater are known to be relatively safe alternatives to seawater. When the relatively high concentration of NaCl is lowered, saline groundwater could be a rich and natural source of various minerals, such as Mg^{2+}, K^{+}, and Ca^{2+} (Kim et al. 2003; Ha et al. 2013). In our future research, we plan to focus our attention on magnesium ion (Mg^{2+}) from deep seawater and saline groundwater. This is because Mg^{2+} is a mineral component that has been reported to have beneficial effects on various cardiovascular diseases such as hypertension which is key of other cardiovascular diseases (Kim et al. 2003; Ha et al. 2014; Kim et al. 2016). Low serum concentrations of magnesium ion were related to an increased risk of cardiovascular diseases (Kupetsky-Rincon and Uitto 2012). Additionally, hypomagnesemia due to low serum magnesium concentration is also associated with insulin resistance and diabetes mellitus (Sales and Pedrosa Lde 2006). Indeed, sufficient magnesium intake is known to reduce the development of diabetes mellitus (Joo et al. 2016). Recently, some research groups have found that deep seawater elicits positive effects on diabetes mellitus (Ha et al. 2013) and inhibits adipogenesis (Ha et al. 2014). Thus, due to the modern life style that increases the risk of metabolic syndrome and

Table 2 Skin irritation evaluation after treatment with Jeju saline groundwater*

	Control site								Test site							
	Erythema and Eschar				Edema				Erythema and Eschar				Edema			
	Intact		Abraded		Intact		Abraded		Intact		Abraded		Intact		Abraded	
Time (h) Rabbit #	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*All skin irritation scores were 0 and were checked after 24 and 72 h. The observations were made regarding erythema, eschar, and edema. No symptoms or irritations were observed in the intact and abraded sites

cardiovascular disease, further study about Mg^{2+} in saline groundwater could be helpful (Seo et al. 2015).

As Jeju saline groundwater is a mixture of fresh water and seawater, it shares something in common with deep seawater. Specifically, their mineral compositions are very similar. Thus, Jeju saline groundwater is expected to have similar effects on diabetes mellitus and adipogenesis as deep seawater. We expect that Jeju saline groundwater will be an abundant source of magnesium and other important minerals. In both eye and skin irritation tests, no degree of irritation was caused by Jeju saline groundwater. These results suggest that Jeju saline groundwater has no toxicity toward the eye and skin. In order to further verify the safety of Jeju saline groundwater, other tests need to be conducted to help demonstrate its usefulness in commercial applications, such as drink, food, and cosmetics.

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Disclosure None of the authors of this study has any financial interest or conflict with industries or parties.

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