Eye and skin irritation tests using deep sea water-extracted minerals

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Received: 13 May 2016 / Accepted: 23 May 2016 / Published Online: 30 September 2016
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Abstract Deep sea water (DSW) contains many inorganic materials and minerals, which are extracted for use in diverse fields. However, before application, safety tests should be performed. In the present study, two DSW-extracted minerals were analyzed: calcium (Ca) and magnesium (Mg). Eye and skin irritation tests of DSW-extracted Ca and Mg were performed according to the guidelines of the Ministry of Food and Drug Safety of Korea. These two types of mineral extracts caused no symptoms of eye and skin irritation when compared to the control. These results suggest that DSW-extracted minerals caused no irritation in the eyes and on the skin, and could be safely applied to these areas.

Keywords Calcium · Deep sea water · Eye irritation · Magnesium · Skin irritation

Introduction

Recently, metabolic syndrome has been a major problem globally and its incidence has steadily increased, resulting from surplus energy intake and sedentary lifestyles. Cardiovascular disease and

diabetes are associated with metabolic syndrome through metabolic disorders resulting from several interrelated risk factors, such as high blood pressure, increased triacylglycerol levels, low high-density lipoprotein cholesterol levels, and obesity. Prevention and treatment of metabolic syndrome are important. The available metabolic syndrome therapies include pharmacological approaches and the improvement of dietary habits. However, the major problem with these available metabolic syndrome therapies is a relatively high rate of adverse side effects (Ha et al. 2013; Kaur 2014). Therefore, the demand for natural compounds with no side effects and certified safety measures to treat metabolic syndrome is increasing.

DSW is readily available natural resource compared to other natural products. And it also has stability and safety (Ha et al. 2013). There are a lot of minerals, such as calcium (Ca), magnesium (Mg), vanadium (V), zinc (Zn), and potassium (K). Many studies have shown that DSW can be effectively used to treat diabetes, obesity, hyperlipidemia, and hypertension (Sheu et al. 2013; Ha et al. 2014b; Li et al. 2014; Yang et al. 2014). Mg supplementation improves myocardial metabolism and inhibits myocardial cell death. Thus, it enhances vascular tone, cardiac output, and afterload, and reduces cardiac arrhythmias (Lin et al. 2010; Pachikian et al. 2010). Another study indicated that Mg supplementation improves lipid metabolism and reduces vulnerability to oxygen-derived free radicals (Dickens et al., 1992; Shechter, 2010). Ca supplementation improves serum cholesterol levels, hypertension, diabetes, and weight regulation (Villegas et al. 2009; Jones et al. 2013). It has been reported that Ca supplementation might ultimately reduce the occurrence of cardiovascular disease and has been recommended for elderly people and postmenopausal women to maintain bone health (Li et al. 2012).

All the aforementioned studies indicate that DSW has been studied for the treatment of metabolic disorders. However, Mg and Ca mineral extracts from DSW have not been investigated for use as anti-metabolic disease agents. However, prior to such investigations, it is necessary to determine the safety of these mineral extracts. In the present study, the eye and skin safety of

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DSW-extracted minerals was demonstrated using eye and skin irritation tests of Notification “2015–82” of the Ministry of Food and Drug Safety of Korea.

Materials and Methods

Animals

To determine eye irritation caused by DSW-extracted minerals, male New Zealand white rabbits were used (Orient Bio, Seongnam, Korea). The body weight of the rabbits was > 1.8 kg. They were housed at 20 ± 2 °C and 50 ± 5 % humidity, and under a 12-h light-dark cycle. They were supplied free access to food pellets (Cargill Agri Purina, Seongnam, Korea). All the animal experiments in this study were approved by the Korea University Institutional Animal Care and Use Committee (KUIACUC-2016-30).

Samples

DSW samples were taken from the deep sea around Goseong, Gangwon Province, Korea. Mineral extracts (Ca and Mg) in powder form were manufactured and provided by the Korea Research Institute of Ships and Ocean Engineering (Daejeon, Korea). Ca mineral extracts for the Ca group were composed of 11.38 wt.% CaCO_3 , 49.50 wt.% CaSO_4 , 21.50 wt.% CaCl_2 , 5.20 wt.% MgCl_2 , 6.68 wt.% MgSO_4 , 2.23 wt.% MgO , and 2.53 wt.% NaCl , thus containing 82.38 wt.% Ca salt. Mg mineral extracts for the Mg group were composed of 48.95 wt.% MgCl_2 , 10.50 wt.% MgSO_4 , 19.65 wt.% MgO , and 23.13 wt.% NaCl , thus containing 79.10 wt.% Mg salt. The mineral extract powder was diluted to 0.5 g/mL in saline solution and the diluent was sonicated for 30 min.

Sample application for the eye irritation test

Before the eye irritation test, we determined that the eyes were free of defects and irritation. The eyes were stained with 5% sodium fluorescein (Sigma-Aldrich, St. Louis, MO, USA) and then checked under blue light at 495 nm. Green light was emitted at 520 nm from corneal lesions. After 24 h, the eyes were examined according to the Food and Drug Safety Guideline of the Ministry of Food and Drug Safety of Korea. Two DSW-extracted minerals were tested as follows: The rabbits were divided into 4 groups; washed Ca (WCa), unwashed Ca (UCa), washed Mg (WMg), and unwashed Mg (UMg). Ca and Mg groups comprised 11 rabbits, which were divided into 4 washed (the eyes were washed with saline solution after treatment with DSW-extracted minerals) and 7 unwashed rabbits

Observation and evaluation of the eye irritation test

Scores were checked on d 1, 2, 3, 4, and 7, and the photographs were taken on d 0, 3, 4, and 7, respectively, after applying mineral extract to the eyes. The degree of eye irritation was evaluated according to scoring charts of the Ministry of Food and Drug

Safety (Chungbuk, Korea) guidelines. The scoring charts included those for cornea, iris, and conjunctiva irritation.

Sample application for the skin irritation test

For the skin irritation test, the hair from the backs of the rabbits was removed. We marked four squares (2.5×2.5 cm) in the glabrous area with a purple skin marker. The needle of a syringe was used to cause an abrasion in the inner area of two squares on the left side. One milliliter of each mineral extract, Ca and Mg, was applied to one intact and one abraded area. Seven rabbits were used for each Ca and Mg treatment group. After treatment, the site was covered with sterile gauze and fixed with nonirritant tape, and stocking rack was placed on the rabbits to avoid detachment of the sterile gauze.

Observation and evaluation of the skin irritation test

After 24 and 72 h of treatment, the degree of the skin irritation was checked by the scoring charts of the Ministry of Food and Drug Safety guidelines. We took photographs of the back skin after 48 h of mineral-extract treatment.

Results and Discussion

DSW inhibits hyperglycemia and improves glucose intolerance by regulating the expression of genes associated with glycometabolism. In addition, the expression of glycogenolysis-, gluconeogenesis-, and genes that were related with glucose oxidation in the livers of diabetes mice was downregulated by DSW treatment. In contrast, the expression of glucose uptake-related genes in the skeletal muscle of diabetes mouse was upregulated by DSW treatment. Thus, LKB1, AMPK, and mTOR activation was restored by DSW (Ha et al. 2014b). Another study indicated that DSW could attenuate hypertension and decrease lipid accumulation by the activation of AMPK/ACC signaling (Sheu et al. 2013). DSW has also been studied in the treatment of metabolic disorders. However, to our knowledge, the DSW-extracted minerals, Mg and Ca, have not previously been investigated for use as anti-metabolic disease agents.

Mg supplementation improves myocardial metabolism and inhibits myocardial cell death by reducing the inflammatory response. It reduces cardiac arrhythmias and prevents intra-mitochondrial Ca accumulation, thereby inducing improved vascular tone, afterload, and cardiac output (Shechter 2010). Hypertension is induced by vascular endothelial dysfunction, which is a risk factor for cardiovascular events. Vascular endothelial function was significantly improved by Mg supplementation (Shechter et al. 2003). Diabetes mellitus is associated with Mg deficiency, which has an important role in the diabetic etiology. Therefore, Mg supplementation could prevent diabetes mellitus (Seelig et al. 2003).

Villegas et al. (2009) and Jones et al. (2013) reported that Ca

Table 1 Eye irritation score. The following 4 groups were analyzed: washed Ca (WCa), unwashed Ca (UCa), washed Mg (WMg), and unwashed Mg (UMg)

		Ca mineral												Mg Mineral											
Day	Individual number	Washed				Unwashed								Washed				Unwashed							
		1	2	3	4	9	10	11	12	13	14	15	5	6	7	8	16	17	18	19	20	21	22		
Day 1	Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Iris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0		
	Iris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0		
	Iris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0		
	Iris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0		
	Iris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Conjunctiva	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

supplementation improved serum cholesterol levels, hypertension, diabetes, and weight regulation. Body fat is affected by dietary Ca, which downregulates intracellular Ca²⁺ in adipocytes by decreasing vitamin D and hormone levels, resulting in the modulation of lipid metabolism and triacylglycerol accumulation in adipocytes (Yoda et al. 2015). Additionally, another study determined that a combination of Mg and Ca accelerates skin barrier recovery (Denda et al., 1999). However, regardless of these reports, safety issues of these minerals have not yet been confirmed.

The Ministry of Food and Drug Safety of Korea has recommended the submission of data related to safety tests of DSW-extracted minerals for the approval of these minerals as functional agent ingredients for pharmacological therapies. To determine the safety of the DSW-extracted minerals, we performed eye and skin irritation tests on rabbits to ensure that Mg- and Ca-mineral extracts were not toxic. Regardless of the relatively high mineral concentration applied, no symptoms or irritation were induced by the DSW-extracted minerals in the cornea, iris, or conjunctiva (Table 1). There was no difference in irritation score between the control and treated eyes. For the treated eyes, there

Fig. 1 Eye irritation test. The following 4 groups were studied: washed Ca (WCa), unwashed Ca (UCa), washed Mg (WMg), and unwashed Mg (UMg). The control was the eye contralateral to the tested eye. The day immediately before applying DSW-extracted Ca and Mg was designated as Day 0

Table 2 Skin irritation score. Back skin was divided into 4 areas: intact control, abraded control, intact test, and abraded test sites. The intact site was used as the control. Each area was 2.5 cm in width and length

Time (h)	Individual number	Control site								Test site							
		Erythema & Eschar				Edema				Erythema & Eschar				Edema			
		Intact		Abraded		Intact		Abraded		Intact		Abraded		Intact		Abraded	
		24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72
Ca	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mg	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Fig. 2 Skin irritation test. The following 4 sites were studied: intact control, abraded control, intact test, and abraded test sites. DSW-extracted Ca and Mg minerals were tested

was no difference in the irritation score before and after treatments. Additionally, no evident differences were found between the washed and unwashed groups (Fig. 1). Thus, there were no specific symptoms or abnormalities in the eye test. Similarly, in the skin irritation test, no abnormalities were found. There was no erythema, eschar, or edema at the control or treatment sites. Additionally, we observed no difference between the intact and abraded sites (Table 2). No visual phenomena caused by the DSW-extracted minerals were observed on the back skin of the rabbits, as shown in Fig. 2.

The present experiments aimed to investigate the safety of DSW-extracted minerals by performing eye and skin irritation tests. The results indicated that the two mineral products, which

were prepared using Mg and Ca from DSW, are not toxic to the eyes or skin of rabbits. However, DSW-extracted Mg and Ca should be studied further to obtain additional toxicity and safety data before their application in metabolic disorder therapies.

Acknowledgments This work was supported by a grant (PJ010529) from the Ministry of Oceans and Fisheries, Republic of Korea.

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