Single- and repeated-dose oral toxicity tests of deep sea water mineral extracts in ICR mice

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Abstract Deep sea water (DSW) is located 100 to 500 m below the sea surface. DSW is widely used in various fields, and is an important source of minerals that can be used to treat mineral deficiency. In the present study, the oral toxicity of DSW-mineral extracts was determined using single-dose and 14-day repeated dose oral toxicity tests in ICR mice. For the single-dose oral toxicity tests, mineral extracts of magnesium (Mg) and calcium (Ca) at doses of 0, 6, 270, 810, and 1,350 mg/kg, respectively, were orally administered to mice once at the beginning of the experiment, and the mice were observed for 14 days. For the 14-day repeated dose oral toxicity tests, Mg and Ca mineral extracts at doses of 0, 3, 135, 405, 675 mg/kg, respectively, were orally administered to mice daily, and the mice were observed for 14 days. Various tests were performed including visual observation; analysis of relative organ weight, food intake, and organ weight; biochemical analysis, and histopathology. The results indicated that mortality and changes in appearance were not observed among differentially administered groups of male and female ICR mice during the experimental period. Differences in body weight gain, food intake, organ weight, and histopathology parameters were not significant between the control and mineral-administered

groups. Some results of the biochemical analyses were significantly different, but showed no specific tendencies. Overall, no evidence of toxicity was observed from the oral administration of DSW extracts of Ca and Mg in ICR mice.

Keywords Calcium · Deep sea water · Magnesium · Mineral extract · Toxicity

Introduction

Deep sea water (DSW) is located 100–500 m below the sea surface. DSW moves continually in a cycle throughout the ocean, and accounts for about 90 % of the whole sea volume (Miyamura et al. 2004). DSW has been used in therapies for the treatment and prevention of various diseases, such as hyperlipidemia, atherosclerosis, hypertension, and dermatitis (Yoshioka et al. 2003; Hataguchi et al. 2005; Li et al. 2014). Compared with surface sea water, DSW is cleaner and more hygienic, with abundance of minerals including magnesium (Mg^{2+}), calcium (Ca^{2+}), and potassium (K^+) ions (Katsuda et al. 2008; Li et al. 2014).

Minerals are essential factors for the health and appropriate development of humans (Ayaz et al. 2006). Humans must ingest minerals in the diet, as they are essential nutrients and cannot be synthesized. Minerals are classified into two groups: macro and micro minerals, of which > 100 mg and < 100 mg, respectively, are needed daily. Classified according to this standard, macro minerals include Ca, phosphorus (P), sodium (Na), K, Mg, sulfur (S), and chlorine (Cl), and micro minerals include iron (Fe), zinc (Zn), nickel (Ni), vanadium (V), and silicon (Si) (Farrell and Nicoteri 2007). Ca, like P and Mg, is an important element for the skeleton, but also has a role in blood coagulation, inhibition of muscle excitability, and the facilitation of neuron stimulus transmission (Harrison and Harrison, 1950). Mg plays a role in the physiological regulation of blood pressure, and has therapeutic

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effectiveness in the treatment of pre-eclampsia (Jain et al. 2010).

Modern society is susceptible to a lack of minerals caused by poor eating habits, including an excessive intake of energy from food and poor intake of micronutrients such as vitamins and minerals. Therefore, micronutrients should be supplemented to avoid deficiencies. As mentioned above, because DSW is an important source of minerals, there have been many attempts to use DSW for such purposes. However, limited research has been performed to examine the *in vivo* toxicity of DSW to ensure it is safe for consumption. In the present study, we will determine the *in vivo* toxicity of minerals extracted from DSW from the East Sea, Korea, for use in commercial products. The purpose of the experiment is to ensure that the mineral extracts from DSW are physiologically safe for common consumption to supplement mineral deficiencies.

Materials and Methods

Animals

Five-week-old male and female ICR mice were obtained from the Shizuoka Laboratory Center (Seoul, Korea), and were acclimated for one week. Animals were housed in facilities of the Laboratory Animal Research Center, Korea University College of Medicine. A total of 46 male and 46 female mice were used for each of the single-dose and repeated-dose oral toxicity tests. Each single and repeated-dose oral toxicity experiment had 9 groups. This research was approved by the Korea University Institutional Animal Care and Use Committee (IACUC).

Deep sea water mineral extracts

Mineral extracts powder (Ca, Mg) from DSW obtained around Goseong (Gangwon Province, Korea) were manufactured and provided by the Korea Research Institute of Ships and Ocean Engineering (Daejeon, Korea). Ca mineral extracts 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 , and thus contained 82.38 wt.% of Ca salt. Mg mineral extracts were composed of 48.95 wt.% MgCl_2 , 10.50 wt.%

MgSO_4 , 19.65 wt.% MgO , and 23.13 wt.% NaCl , and thus contained 79.10 wt.% of Mg salt.

Single oral toxicity tests

Single oral toxicity studies were performed according to the guidelines of the Ministry of Food and Drug Safety of Korea. Each group consisted of 5 male and 5 female mice. Mineral extracts of Mg and Ca were diluted with distilled water to produce mineral concentrations of 0.6, 27.5, 81.0, 135 g/L, respectively, and sonicated for 30 min. All groups were administered single-oral doses at 10 mL of the diluted mineral/kg of body weight. The animals were observed during the first 30 min and once daily for 14 days. Body weight was measured every 3 days. After 14 days, the animals were sacrificed, and their heart, kidney, spleen, liver, ovary and testis were weighed (Lee et al. 2006).

Repeated-dose oral toxicity tests

Repeated-dose oral toxicity studies were also performed according to the guidelines of the Ministry of Food and Drug Safety of Korea. As above, each group contained 5 male and 5 female mice and all mice was divided into 9 groups. All groups were orally administered repeated doses at 10 mL of the diluted mineral/kg of body weight every day for 14 days. Gross observations were conducted daily, and body weight was measured once every 3 days. After 14 days, the animals were sacrificed, and their heart, kidney, spleen, liver, ovary and testis were weighed. The total protein, albumin, globulin, glucose, blood urea nitrogen (BUN), alanine transaminase (ALT), aspartate amino transferase (AST), P, Ca, and Mg were measured by Eone Laboratories (Incheon, Korea). For histopathology, the organs of 405 mg/kg Mg and 405 mg/kg Ca groups were fixed in 10 % neutrally buffered formalin and embedded in paraffin after 18 h. Slices of 3–4 μm thickness were prepared, and stained with hematoxylin and eosin (H&E) (Beppu et al. 2009).

Statistical analyses

The differences between the control and treatment groups were determined by an independent sample t-test.

Table 1 Body weight gain in mice administered minerals orally for 14 days

Mean $\pm$ SD	Ca mineral					Mg mineral			
	0 mg/kg	3 mg/kg	135 mg/kg	405 mg/kg	675 mg/kg	3 mg/kg	135 mg/kg	405 mg/kg	675 mg/kg
Female									
Initial weight (g)	26.60 $\pm$ 1.52	26.80 $\pm$ 0.84	26.40 $\pm$ 1.14	27.20 $\pm$ 0.84	26.83 $\pm$ 2.23	26.60 $\pm$ 1.82	26.60 $\pm$ 1.52	26.20 $\pm$ 1.64	25.50 $\pm$ 1.76
Final weight (g)	29.60 $\pm$ 1.95	29.80 $\pm$ 2.17	29.00 $\pm$ 1.00	30.20 $\pm$ 1.64	31.50 $\pm$ 2.81	29.00 $\pm$ 2.35	29.00 $\pm$ 1.22	29.60 $\pm$ 1.82	30.17 $\pm$ 1.94
Body weight gain (%)	11.25 $\pm$ 2.46	11.34 $\pm$ 9.75	10.02 $\pm$ 6.23	11.01 $\pm$ 4.33	17.53 $\pm$ 7.11	9.02 $\pm$ 4.97	9.19 $\pm$ 5.41	13.05 $\pm$ 4.58	13.92 $\pm$ 4.82
Male									
Initial weight (g)	33.00 $\pm$ 1.87	33.60 $\pm$ 1.82	34.60 $\pm$ 1.52	33.80 $\pm$ 2.39	34.83 $\pm$ 2.04	33.60 $\pm$ 1.82	33.60 $\pm$ 1.52	35.80 $\pm$ 1.79	34.00 $\pm$ 1.55
Final weight (g)	38.60 $\pm$ 2.51	39.40 $\pm$ 2.19	40.80 $\pm$ 2.59	39.20 $\pm$ 2.68	39.50 $\pm$ 3.08	37.60 $\pm$ 3.05	38.60 $\pm$ 0.89	38.60 $\pm$ 2.70	40.00 $\pm$ 2.76
Body weight gain (%)	17.00 $\pm$ 4.70	17.32 $\pm$ 4.45	17.86 $\pm$ 3.23	16.10 $\pm$ 5.60	13.32 $\pm$ 3.97	11.85 $\pm$ 5.54	15.02 $\pm$ 4.44	17.75 $\pm$ 7.01	17.59 $\pm$ 4.65

Fig. 1 Organ weight change in ICR mice during 14 days of repeated oral administration. (A) Female heart, (B) Female kidney, (C) Female spleen, (D) Female liver, (E) Ovary, (F) Male heart, (G) Male kidney, (H) Male spleen, (I) Male liver, (J) Testis. Data are represented as mean $\pm$ SD ($n = 5$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group (student's t test)

Results

Single-dose oral toxicity

Mortality and change in appearance were not observed among groups of male and female ICR mice treated with different concentrations of Mg and Ca minerals during the experimental period of 14 days. There was no significant difference in body weight gain between the control and administered groups. The

weights of the liver, heart, kidney, spleen, ovary, and testis between the control and administered groups were also not statistically different during 14 days (data not shown).

Repeat-dose oral toxicity

Mortality and change in appearance were not observed among groups of male and female ICR mice treated daily for 14 days with different Mg and Ca concentrations. There was no significant difference in body weight gain between the control groups and administered groups. Initial body weight, final body weight, and body weight gain are shown in Table 1. As shown in Fig. 1, no trend was observed among values of organ weight for the liver, heart, kidney, spleen, ovary, and testis during 14 days.

Table 2 shows the experimental results of serum biochemical parameters. There was no trend observed in the values for total protein, albumin, globulin, glucose, AST, ALT, BUN, P, Ca, and Mg. As shown in Fig. 2, histopathology indicated no damage in the heart, kidney, spleen, or liver of the treated mice.

Fig. 2 Sections of organ were stained with hematoxylin and eosin (H&E-staining) showing the effect of mineral extracts on repeated-dose oral toxicity in ICR mice. (A) Female organ, (B) Male organ

Table 2 Serum biochemical parameters in mice administered minerals orally for 14 days

Mean $\pm$ SD	Ca mineral					Mg mineral			
	0 mg/kg	3 mg/kg	135 mg/kg	405 mg/kg	675 mg/kg	3 mg/kg	135 mg/kg	405 mg/kg	675 mg/kg
Female									
Total protein (g/dL)	5.41 $\pm$ 0.23	5.43 $\pm$ 0.34	5.04 $\pm$ 0.40	5.37 $\pm$ 0.41	5.15 $\pm$ 0.37	5.24 $\pm$ 0.23	5.52 $\pm$ 0.32	5.42 $\pm$ 0.20	5.54 $\pm$ 0.47
Albumin (g/dL)	3.61 $\pm$ 0.20	3.57 $\pm$ 0.25	3.24 $\pm$ 0.52	3.63 $\pm$ 0.34	3.45 $\pm$ 0.20	3.54 $\pm$ 0.13	3.74 $\pm$ 0.22	3.67 $\pm$ 0.11	3.69 $\pm$ 0.33
Globulin (g/dL)	1.80 $\pm$ 0.12	1.85 $\pm$ 0.16	1.80 $\pm$ 0.23	1.74 $\pm$ 0.12	1.71 $\pm$ 0.19	1.70 $\pm$ 0.14	1.78 $\pm$ 0.11	1.75 $\pm$ 0.13	1.84 $\pm$ 0.16
Glucose (mg/dL)	365.75 $\pm$ 70.09	333.60 $\pm$ 36.55	331.40 $\pm$ 80.24	312.00 $\pm$ 78.81	408.83 $\pm$ 91.08	313.60 $\pm$ 67.81	292.60 $\pm$ 59.54	323.60 $\pm$ 91.51	433.50 $\pm$ 113.12
AST (U/L)	61.50 $\pm$ 16.74	59.20 $\pm$ 22.63	57.00 $\pm$ 13.30	48.40 $\pm$ 7.83	47.83 $\pm$ 8.91	59.60 $\pm$ 22.70	51.60 $\pm$ 8.38	46.40 $\pm$ 4.39	56.33 $\pm$ 9.77
ALT (U/L)	25.25 $\pm$ 6.24	34.40 $\pm$ 31.70	23.40 $\pm$ 13.15	22.80 $\pm$ 3.49	22.17 $\pm$ 3.49	25.00 $\pm$ 10.65	25.00 $\pm$ 7.11	17.00 $\pm$ 1.58*	21.17 $\pm$ 3.49
BUN (mg/dL)	34.60 $\pm$ 13.93	27.58 $\pm$ 6.85	42.06 $\pm$ 24.97	33.30 $\pm$ 13.89	34.00 $\pm$ 8.97	30.58 $\pm$ 8.98	21.16 $\pm$ 7.97	29.34 $\pm$ 6.07	40.33 $\pm$ 8.51
Phosphorus (mg/dL)	12.31 $\pm$ 1.79	11.87 $\pm$ 1.34	11.32 $\pm$ 1.13	12.34 $\pm$ 2.10	12.41 $\pm$ 1.62	14.24 $\pm$ 2.27	10.15 $\pm$ 4.91	11.66 $\pm$ 1.31	13.23 $\pm$ 3.27
Calcium (mg/dL)	11.80 $\pm$ 0.80	11.48 $\pm$ 0.19	11.00 $\pm$ 0.30	10.90 $\pm$ 0.28*	11.10 $\pm$ 0.42	10.82 $\pm$ 0.29*	9.24 $\pm$ 4.29	10.82 $\pm$ 0.31*	11.62 $\pm$ 1.83
Magnesium (mg/dL)	2.93 $\pm$ 0.30	2.85 $\pm$ 0.22	2.73 $\pm$ 0.56	2.73 $\pm$ 0.20	2.56 $\pm$ 0.16	2.85 $\pm$ 0.17	2.61 $\pm$ 0.20	10.82 $\pm$ 0.21	2.95 $\pm$ 0.66
Male									
Total protein (g/dL)	5.65 $\pm$ 0.54	5.45 $\pm$ 0.46	5.81 $\pm$ 0.28	5.63 $\pm$ 0.36	6.00 $\pm$ 0.66	5.74 $\pm$ 0.20	4.89 $\pm$ 1.34	5.15 $\pm$ 0.15	6.02 $\pm$ 0.50
Albumin (g/dL)	3.36 $\pm$ 0.28	3.30 $\pm$ 0.38	3.50 $\pm$ 0.16	3.40 $\pm$ 0.19	3.61 $\pm$ 0.22	3.51 $\pm$ 0.19	2.93 $\pm$ 1.05	3.11 $\pm$ 0.12	3.66 $\pm$ 0.30
Globulin (g/dL)	2.29 $\pm$ 0.28	2.14 $\pm$ 0.10	2.31 $\pm$ 0.15	2.23 $\pm$ 0.21	2.39 $\pm$ 0.47	2.23 $\pm$ 0.12	1.96 $\pm$ 0.30	2.03 $\pm$ 0.07	2.36 $\pm$ 0.23
Glucose (mg/dL)	465.75 $\pm$ 88.36	458.40 $\pm$ 140.77	537.60 $\pm$ 110.89	399.40 $\pm$ 37.63	469.00 $\pm$ 107.46	407.50 $\pm$ 95.43	349.20 $\pm$ 81.75	396.80 $\pm$ 87.21	473.33 $\pm$ 63.90
AST (U/L)	61.00 $\pm$ 18.09	50.80 $\pm$ 13.66	40.40 $\pm$ 2.88	58.80 $\pm$ 14.43	56.67 $\pm$ 23.58	45.25 $\pm$ 8.73	37.80 $\pm$ 6.72	38.80 $\pm$ 4.32*	46.50 $\pm$ 9.40
ALT (U/L)	33.75 $\pm$ 5.85	25.80 $\pm$ 5.89	27.80 $\pm$ 5.26	32.80 $\pm$ 6.57	30.83 $\pm$ 3.37	32.00 $\pm$ 8.68	25.80 $\pm$ 10.18	28.20 $\pm$ 5.26	31.00 $\pm$ 8.79
BUN (mg/dL)	42.03 $\pm$ 9.68	34.42 $\pm$ 3.63	33.56 $\pm$ 6.58	37.68 $\pm$ 12.39	35.95 $\pm$ 6.07	37.90 $\pm$ 10.46	36.92 $\pm$ 7.91	37.62 $\pm$ 4.29	39.30 $\pm$ 8.73
Phosphorus (mg/dL)	10.29 $\pm$ 0.71	10.47 $\pm$ 1.04	10.42 $\pm$ 1.13	9.92 $\pm$ 1.44	11.28 $\pm$ 0.99	9.61 $\pm$ 0.98	8.74 $\pm$ 0.54*	8.51 $\pm$ 1.71	10.93 $\pm$ 2.88
Calcium (mg/dL)	11.83 $\pm$ 0.59	11.28 $\pm$ 0.47	11.62 $\pm$ 0.42	11.70 $\pm$ 0.67	11.72 $\pm$ 0.86	11.50 $\pm$ 0.85	10.54 $\pm$ 0.25	11.16 $\pm$ 0.54	11.90 $\pm$ 1.16
Magnesium (mg/dL)	2.54 $\pm$ 0.33	2.35 $\pm$ 0.22	2.47 $\pm$ 0.08	2.51 $\pm$ 0.30	2.61 $\pm$ 0.46	2.58 $\pm$ 0.42	2.31 $\pm$ 0.32	2.32 $\pm$ 0.17	2.70 $\pm$ 0.29

Discussion

Toxicity studies during the drug development phase are necessary to predict human toxicity aspects of chemicals based on results obtained from test animals, and to monitor the toxicity symptoms in patients. The results of the present study can be utilized to establish safe doses for clinical trials, since the repeated-dose toxicity study of DSW extracts was performed in accordance with the Drug Toxicity Testing Standards of Notification (No.2012-64) of the Ministry of Food and Drug Safety of Korea. Both male and female ICR mice were orally administered doses of 0, 6, 270, 810, 1350 mg Mg or Ca mineral extract/kg of body weight in the single-dose oral toxicity study. No toxic lesions were found in any ICR mice from all experimental groups that were observed for 14 days (data not shown), in either the single-dose or repeated-dose toxicity tests. Both male and female ICR mice were orally administered doses of 0, 3, 135, 405, 675 mg Mg or Ca mineral extract/kg of body weight in repeated-dose oral toxicity study. During the 14 days, no expression of toxic symptoms with Mg and Ca were observed regarding mortality, body weight, and

autopsy findings (Figs. 1, 2; Table 1). Thus, mortality and change in appearance were not observed in any experimental group.

For the repeated-dose oral toxicity study, the t-test indicated that organ weights of some female mice were significantly different from that of the control group: kidneys for 3 mg/kg Ca and 405 mg/kg Mg; livers for 135 and 405 mg/kg Ca, and 3, 135, and 405 mg/kg Mg; ovaries for 405 mg/kg Ca, and 135 mg/kg Mg. In addition, organ weights of some male mice were significantly different from that of the control group: heart for 3, 135, 405, 675 mg/kg Ca, and 675 mg/kg Mg; spleen for 135, and 405 mg/kg Ca, and 3, 135, 405, and 675 mg/kg Mg; liver for 3, 135, and 675 mg/kg Ca, and 135 and 405 mg/kg Mg; 3 and 405 mg/kg Ca. However, the change of each organ weight compared to the control showed no specific trend with different mineral concentrations. In addition, no statistical significance in each organ weight was found between Mg and Ca mineral groups.

Some groups showed a statistical significance in the values of the serum biochemical parameters, but no specific trend was observed. Histological examination of the mineral extract-treated samples of liver, heart, kidney and spleen showed some abnormal

morphological characteristics in all groups but no specific trend was observed (Fig. 2). In addition, no specific toxicity lesions were observed in the histological analyses.

These results indicated that single and repeated-dose oral toxicity analyses of DSW-mineral extracts (Ca, Mg) in male and female ICR mice did not show indications of acute toxicity, such as variations in mortality, body weight, general symptoms, serum biochemical parameters, and histopathology. Together, the results showed that the DSW-mineral extracts (Ca, Mg) were not toxic for single-dose and repeated-dose oral treatments in ICR mice.

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