Single and repeated oral dose toxicity tests of saline groundwater in ICR mice

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Abstract Saline groundwater (SGW) is the underground saline water obtained from basalt layer through which seawater has infiltrated. SGW contains more than 10,000 mg/L dissolved solid, the value of which is less than that of seawater. As part of its safety test, single and repeated oral dose toxicity tests were conducted with male and female ICR mice for 14 days. In single oral dose test with dosage of 10, 30, and 50 mL/kg, no gross changes in appearance or mortality were observed. In repeated oral dose test with dosage of 8, 14, and 20 mL/kg, no significant changes in mortality or weights of body and organ were observed. Additional analysis of serum biochemical parameter and histopathology also indicated no meaningful change during the tests. When taken all together, these results show that no toxicity of SGW could be found with single and repeated toxicity tests. However, for final conclusion of safety, further toxicity studies need to be performed with animal and human subjects.

Keywords Mineral · Oral toxicity · Safety test · Saline groundwater

Introduction

Minerals are one of the vital sources, since they cannot be synthesized. Thus, they should be taken in the diet [1]. Sodium ion (Na⁺) is an abundant cation in extracellular fluid. It is also involved in the maintenance of plasma osmotic pressure and regulates blood pressure, solute transport, and synaptic transmission in cooperation with potassium ion (K⁺) through the Na⁺–K⁺ pump [2, 3]. Calcium ion (Ca²⁺) acts as a second messenger with cAMP and is used as a main signaling molecule in most muscles [4–6]. Magnesium ion (Mg²⁺) is a well-known cofactor in various enzyme reactions and an essential factor in protein synthesis, mitochondrial integrity, or improving glycerol control [7, 8]. Low intake of those minerals can induce mineral imbalance of the body and cause various nutrition deficiency symptoms, such as anemia, ataxia, and nausea [9]. On the other hand, over-consumption of minerals can induce hypercalciuria, hypercalcemia, hypotension, or diarrhea [9]. There is also a report that deficiency of some minerals, such as Mg²⁺, brings about lung cancer [10]. Saline groundwater (SGW) is obtained from basalt layer into which seawater has infiltrated [11]. Unlike common groundwater of inland, SGW contains more than 10,000 mg/L dissolved solid but less than that of seawater [12]. SGW is a result of seawater infiltration through basalt layer and the mixing of seawater and groundwater so that it has both the properties of seawater and groundwater [11]. SGW have a characteristic composition of minerals such as Na⁺, K⁺, Ca²⁺, and Mg²⁺ [11]. Despite this special character and usefulness, the safety tests of SGW as a food source have rarely been studied. Previously, we performed several toxicity tests, including eye and skin irritation test.

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[13]. Here, we conducted single and repeated oral dose toxicity tests of SGW employing ICR mice to prove no toxicity of SGW by oral treatment.

Materials and methods

Animals

Five-week-old male and female ICR mice were purchased from Shizuoka Laboratory Center (Seoul, Korea) and acclimatized to the test facility for a week. ICR mice were housed in the Gyerim Experimental Animal Resource Center of Korea University (Seoul, Korea). The facilities were maintained under 12-h light–dark cycle with 23 ± 2 °C temperature and 55 ± 5% humidity. After acclimation, the animals were divided into several groups on their initial body weight basis. All animals were given access to food and water ad libitum. This research was performed in accordance with permit of the Korea University Institutional Animal Care and Use Committee (KUIACUC-2016-105).

Sample

SGW sample and its information of ion concentration (hardness) were provided from Jeju TechnoPark, Jeju, Korea. Ion concentrations (hardness) of SGW sample were 10,500 mg/L for sodium ion (Na\(^+\)), 1240 mg/L for magnesium ion (Mg\(^{2+}\)), 451 mg/L for potassium ion (K\(^+\)), 372 mg/L for calcium ion (Ca\(^{2+}\)), 22,000 mg/L for chloride ion (Cl\(^-\)), 2990 mg/L for sulfate ion (SO\(_4^{2-}\)), 1.05 mg/L for fluoride ion (F\(^-\)), 70.1 mg/L for bromide ion (Br\(^-\)), and 4.38 mg/L for boron ion (B\(^{3+}\)). Thus, the hardness of Jeju SGW employed is about 338,000 mg/L.

Single oral dose toxicity tests

After one-week acclimation, ICR mice were divided into four groups, each of which consisted of five male or female ICR mice. Each group included a control group. After gross observation, each experimental group was orally administered with SGW samples once at concentrations of 10, 30, and 50 mL/kg, respectively. Gross appearance and mortality were observed every day for 30 min, before measuring food intake. The amount of food intake was measured every day, and body weight was measured every 3 days. After the experiments, all groups were killed and body weight and their organ weights of heart, kidney, spleen, and liver were measured.

Repeated oral dose toxicity tests

Each group consisted of five male and female ICR mice. Each experimental group was orally administered with SGW samples every day at concentrations of 8, 14, and 20 mL/kg, respectively, after gross observation. The gross appearance and food intake were observed daily for 30 min, and the body weight was measured every 3 days. After 14-day experiment, all animals were killed and body weight was measured. Their blood samples were collected, and weights of heart, kidneys, spleen, and liver were measured after removal. Blood serum was collected from whole blood by centrifugation at 12,000×g for 30 min. The total protein, albumin, globulin, glucose, total cholesterol, aspartate amino transferase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), creatinine, uric acid, P, Ca\(^{2+}\), Mg\(^{2+}\), phospholipid, triglyceride, and free fatty acid were analyzed by Eone Laboratories (Incheon, Korea). Meanwhile, the removed organs were weighed to be sectioned for histopathology. Each organ was fixed in 10% buffered formalin to be embedded in paraffin. After sectioning, hematoxylin and eosin (H&E) were used for staining [14, 15]. All data analysis was conducted using Student’s t test.

Results

Saline groundwater has the characteristics of both deep seawater and groundwater. In addition, it has abundant minerals that are essential for body ion balance. We have performed single and repeated oral dose toxicity tests to make sure of the safety and toxicity of SGW. During 14 days for single oral dose toxicity tests, no gross change in appearance was observed among the administered groups (10, 30, and 50 mL/kg), compared to the control group. While the body weight of ICR mice continuously increased during the experiment, the administered groups had no statistical difference in body weight compared to the control group. There was also no statistical difference in relative organ weights (% of body weight) of the heart, spleen, kidneys, and liver among the administered groups with a few exceptions as follows: Female heart and liver of the 50 mL/kg group had a statistically significant decrease \((p < 0.05)\), while male liver of the 50 mL/kg group had a statistically significant decrease \((p < 0.01)\), compared to the control group (data not shown).

In repeated oral dose toxicity tests, no gross change in appearance was observed among female and male ICR mice groups during the experimental period of 14 days with various concentrations of SGW. In addition, no statistical difference in final body weight (g) or body weight gain (%) has been found with the three tested groups (8, 14,
and 20 mL/kg), compared to control (Table 1). When relative organ weights (% of body weight) of the heart, spleen, kidneys, and liver were compared to the control, no statistical difference was observed among the administered groups with two exceptions as follows: Female liver of the 14 mL/kg group and male liver of the 20 mL/kg groups had a statistically significant change ($p < 0.05$), compared to the control (Figs. 1, 2). In the serum parameter analysis, the values of administered groups showed no statistical difference from those of the control except few exceptions (Table 2). The histopathological data also have shown no symptom of organ damage with the administered groups in comparison with the control (Fig. 3).

Table 1  Body weight gain during oral administration of SGW in repeated oral dose toxicity test for 14 days

|       | Female |       |       |       | Female |       |       |       |
|-------|--------|-------|-------|-------|--------|-------|-------|-------|
| Mean ± SD | Con | 220  | 390  | 560  | Con  | 220  | 390  | 560  |
| Initial weight (g) | 27.86 ± 1.38 | 27.86 ± 1.37 | 27.84 ± 1.17 | 27.84 ± 1.18 | 32.96 ± 0.48 | 33.02 ± 0.52 | 33.00 ± 0.63 | 33.00 ± 0.53 |
| Final weight (g) | 31.44 ± 1.27 | 30.37 ± 1.38 | 30.35 ± 1.31 | 30.27 ± 1.53 | 37.18 ± 0.90 | 35.82 ± 1.55 | 36.58 ± 1.03 | 38.23 ± 1.05 |
| Body weight gain (%) | 12.93 ± 3.53 | 9.77 ± 1.39 | 9.03 ± 2.78 | 8.69 ± 2.66 | 12.83 ± 5.40 | 8.53 ± 10.42 | 10.81 ± 3.37 | 15.85 ± 5.75 |

Data are represented as mean ± SD ($n = 5$). No statistical difference for each body weight item has been found between control and the treated groups.
Discussion

Gross changes in appearance and body weight are significant parameters to decide the degree of the sample toxicity. In single and repeated oral dose toxicity tests, no gross change in appearance was observed among female and male ICR mice groups for 14 days with various concentrations of SGW. Although there were some statistical deviations from general trends in single and repeated oral dose toxicity tests as mentioned above, the overall extent of change was not dependent on the administered concentrations of SGW. This means that the relative organ weight described above had no trend of change with the increase in SGW. The results obtained above with single and repeated oral dose toxicity tests have shown that no toxicity is observed with SGW.

Serum biochemical parameter analysis is performed to estimate the individual physical condition of the experimental subject. The liver plays critical roles in the metabolisms of fat, protein, and carbohydrate [16]. It is also involved in both the maintenance of blood glucose level and the synthesis of bile and lipoprotein. ALT and AST are liver-associated enzymes, the values of which indicate the leakage concentration level of hepatic enzymes into blood circulation [17]. Thus, elevated levels of ALT and AST could be evidence of injured liver [16, 18]. It appeared that although in a few cases the relative liver organ weight of female and male groups had statistical difference compared to the control, such kind of statistical change was not systematically observed for either single or repeated oral dose toxicity tests. In addition, the level of ALT and AST for the administered groups showed no statistical difference compared to the control (Table 2), with the exception of female administered groups at which the level of ALT was much lower than control, indicating the healthier liver condition of the administered groups. It has been reported that increased levels of BUN and creatine reflect a higher risk of heart failure [19, 20]. In the serum parameter analysis of BUN and creatine, the values of administered groups showed no statistical difference from those of the control. This kind of phenomenon was also observed with the other serum parameters. Although there were a few exceptions, there was also no specific trend of the parameter change with the increase in SGW concentrations.

Fig. 2 Change in organ weight in the repeated oral dose toxicity test for A male heart, B male kidney, C male liver, D male spleen. Data are represented as mean ± SD (n = 5). *p < 0.05 compared to the control.
Table 2: Serum biochemical analysis with oral administration SGW in repeated oral dose toxicity test for 14 days

|                     | Female                        | Male                          |
|---------------------|-------------------------------|-------------------------------|
|                     | Control 8 mL/kg 14 mL/kg 20 mL/kg | Control 8 mL/kg 14 mL/kg 20 mL/kg |
| Total protein       | 5.22 ± 0.44 4.78 ± 0.34 4.56 ± 0.45* 4.93 ± 0.45 | 5.07 ± 0.38 4.93 ± 0.52 4.91 ± 0.26 5.20 ± 0.26 |
| Albumin             | 3.60 ± 0.18 3.35 ± 0.19 3.18 ± 0.24* 3.51 ± 0.20 | 3.18 ± 0.08 2.91 ± 0.53 3.11 ± 0.25 3.47 ± 0.22* |
| Globulin            | 1.62 ± 0.26 1.43 ± 0.11 1.38 ± 0.20 1.43 ± 0.23 | 1.89 ± 0.32 2.02 ± 0.54 1.80 ± 0.34 1.73 ± 0.10 |
| Glucose             | 239.00 ± 79.90 272.50 ± 77.07 195.60 ± 29.32 307.80 ± 105.22 | 207.20 ± 71.94 261.60 ± 87.11 296.40 ± 37.62 243.40 ± 41.95 |
| Total Cholesterol   | 89.80 ± 15.41 74.50 ± 10.23 80.20 ± 11.75 82.60 ± 15.60 | 112.60 ± 13.94 94.40 ± 24.15 116.80 ± 8.01 128.20 ± 14.95 |
| AST                 | 99.40 ± 34.64 75.00 ± 16.08 68.00 ± 17.40 68.60 ± 16.81 | 71.80 ± 24.45 96.00 ± 48.53 64.80 ± 22.22 50.60 ± 2.33 |
| ALT                 | 52.40 ± 33.79 25.00 ± 5.66 21.40 ± 2.94 26.80 ± 4.58 | 35.40 ± 6.74 24.00 ± 5.18* 24.80 ± 3.54* 24.20 ± 2.23* |
| BUN                 | 28.52 ± 2.96 24.88 ± 4.16 20.76 ± 2.57** 21.40 ± 2.56** | 28.38 ± 4.17 21.92 ± 2.41* 25.08 ± 2.26 27.72 ± 3.17* |
| Creatinine          | 0.23 ± 0.05 0.17 ± 0.09 0.07 ± 0.06** 0.16 ± 0.07 | 0.11 ± 0.03 0.10 ± 0.03 0.14 ± 0.05 0.11 ± 0.03 |
| Uric acid           | 4.53 ± 1.50 4.68 ± 1.68 2.22 ± 0.47* 5.60 ± 2.20 | 3.90 ± 0.94 5.79 ± 1.49 5.99 ± 0.94* 4.45 ± 1.71 |
| Phosphorus          | 12.14 ± 2.45 10.44 ± 1.94 9.40 ± 1.17 11.97 ± 1.89 | 10.63 ± 2.15 9.87 ± 1.39 10.39 ± 1.07 10.47 ± 2.14 |
| Calcium ion         | 11.08 ± 1.14 11.18 ± 1.39 9.72 ± 0.55 10.28 ± 0.56 | 10.34 ± 0.38 10.74 ± 0.79 10.16 ± 0.54 8.38 ± 3.15 |
| Magnesium ion       | 3.22 ± 0.15 2.92 ± 0.49 2.41 ± 0.12*** 3.35 ± 0.52 | 2.72 ± 0.33 3.04 ± 0.62 2.91 ± 0.14 3.05 ± 0.25 |
| Phospholipid        | 176.00 ± 19.54 144.25 ± 21.51 148.40 ± 18.99 158.00 ± 25.08 | 216.60 ± 24.16 191.00 ± 40.92 232.60 ± 20.19 235.00 ± 22.03 |
| Triglyceride        | 73.40 ± 22.06 54.25 ± 11.01 51.40 ± 11.05 49.60 ± 12.85 | 49.20 ± 6.94 40.00 ± 12.28 49.20 ± 8.70 30.20 ± 4.26*** |
| Free Fatty acid     | 2189.00 ± 837.59 1361.75 ± 282.16 1730.00 ± 211.57 1839.40 ± 514.40 | 1711.60 ± 113.06 1647.20 ± 501.67 1764.20 ± 121.42 1529.40 ± 143.62 |

Data are represented as mean ± SD (n = 5)
*p < 0.05, **p < 0.01, ***p < 0.001 compared to the control
suggesting that the treatment of SGW had no influence on the tested toxicity.

Although the mineral dosage of SGW is lower than that of deep-sea water due to the mixing with inland fresh groundwater, the minerals from SGW may be relatively safer, since SGW is obtained after seawater filtration into basalt layer [11]. Because of this fact, we have one more way to use seawater safely. That is, with effective removal of NaCl, SGW could be employed as a rich and safe source of various minerals, such as Mg, K, and Ca [21, 22]. This fact would make open the way to use SGW directly in any commercial product. For example, it is known that the Mg component among minerals has many beneficial effects for human health. Mg$^{2+}$ is one of the mineral components to treat hypomagnesemia and diabetes mellitus as well as various cardiovascular diseases [13, 21–23]. In another examples, deep-sea water of relatively high Mg$^{2+}$ content had positive effects on diabetes mellitus [22] as well as on the inhibition of adipogenesis [24]. Thus, like deep-sea water, SGW from Jeju could be employed to treat the various diseases mentioned above. We expect that Jeju saline groundwater could be an appropriate source of magnesium and other important minerals. In our future research, we plan to turn our attention to magnesium ion (Mg$^{2+}$) from deep-sea water and saline groundwater. Together with our previous study of eye and skin irritation tests, our present study shows that no degree of toxicity was found with Jeju saline groundwater. For further verification of the safety of Jeju saline groundwater, more safety tests might be performed to maximize the potential of its usefulness in commercial products, including drinks, food, and cosmetics.

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**Fig. 3** Histopathological images of ICR mice organs in the repeated oral dose toxicity test. Sample organ tissues were obtained from mice treated with 20 mL/kg dosage; a female organ, b male organ. Organs were stained with H&E, sectioned, placed on cover glass to be observed with fluorescence upright microscope (Axio Imager M1, Carl Zeiss)
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