Tohoku J. Exp. Med., 2004, **203**, 175-182

Effects of Desalted Deep Seawater on Hematologic and Blood Chemical Values in Mice

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Tsuchiya, Y., Watanabe, A., Fujisawa, N., Kaneko, T., Ishizu, T., Fujimoto, T., Nakamura, K. and Yamamoto, M. *Effects of Desalted Deep Seawater on Hematologic and Blood Chemical Values in Mice*. Tohoku J. Exp. Med., 2004, **203** (3), 175-182 —— Various processed foods and beverages have been manufactured using deep seawater (DSW), desalted DSW (dDSW), and concentrated DSW in Japan. To confirm the safety of dDSW, we investigated hematologic and blood chemical effects of dDSW in mice. The dDSW and desalted surface seawater (dSSW) were diluted to 6.7%, 10%, and 20% with purified water. BALB/c mice were housed for 12 weeks, and administered the diluted dDSW, dSSW, or purified water as a control during the period. The results for dDSW were compared with those for dSSW and purified water. None of the groups of mice showed any clear abnormal growth or behavior; neither did any show signs of illness nor a single case of death during the 12 weeks study. We found no significant differences between the dDSW and control groups in terms of red blood cell count, hemoglobin, hematocrit, white blood cell count, and neutrophil counts, whereas white blood cell and lymphocyte counts were significantly higher in the 10% dSSW group at the end of 4 and 12 weeks than those in the control group. A significantly higher triglyceride level was detected only in the 6.7% dSSW group. Our results show no evidence of acute or subacute effects of diluted dDSW. Effects of diluted dDSW on hematologic and blood chemical values in mice are thought to be similar to those of purified water. This finding suggests that dDSW is as safe as purified water for drinking water. ——— deep seawater; electrodialytic desalination; hematologic values; blood chemical values; mice

Received March 9, 2004; revision accepted for publication May 6, 2004.

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Deep seawater (DSW) utilization technology was developed as the source of heat for ocean thermal energy conversion in the USA in the 1970s. Recently, DSW has been mainly used for the growth of micro algae, macro algae, crustaceans, mollusks, and finfish, or for seawater air conditioning in Hawaii; apart from that, it is used in the field of food processing (e.g.; fermented soybean paste, soybean sauce, confectioneries, bake), and beverage production (e.g.; Japanese sake, beer, drinking water) in Japan.

DSW has some special characteristics that surface seawater (SSW) does not have, such as being very pure, rich in several types of inorganic nutrient salts, and low temperature stability (Sasaki 2001). In fact, no disease-causing bacteria have been detected in DSW collected from the Sea of Japan (Ogawa and Fujimoto 2002). Although some studies have examined the characteristics of DSW (Suzuki 2000; Kimata et al. 2002; Ueshima et al. 2003), the efficacy of DSW has not clearly been determined yet.

To promote the effective utilization of DSW collected from the Sea of Japan, we have been conducting research on applications of DSW in terms of health care and health promotion (Tsuchiya et al. 2002, 2003a,b). Our previous study revealed that serum concentrations of IgG and glucose in mice increased significantly 12 weeks after the administration of the 12% DSW diluted with purified water, compared with those administered purified water. Whereas some studies on the health effects of the diluted DSW as described above are reported, desalted DSW (dDSW) is used widely as drinking water in Japan. Therefore, it is necessary to confirm the safety and health effects of dDSW.

The aim of the present study was to reveal the health effects of dDSW diluted with purified water: specifically, to conduct an acute or subacute toxicity test to confirm the safety of dDSW. We studied the effects of dDSW on hematologic and blood chemical values in mice by administering the dDSW orally ad libitum for 12 weeks. The results of dDSW were compared with those of desalted SSW (dSSW) diluted with purified water, using purified water as a control.

Materials and Methods

Collection of DSW and SSW

We collected DSW and SSW from depths of approximately 300 meters and 5 meters in the Sea of Japan (37.50-38.00°N latitude; 138.30-138.45°N longitude), using the DSW-drawing system developed jointly by HONMA Co. Ltd., and KITAC Corporation (Niigata). Water was gathered in an 18-liter plastic container and sent immediately to our laboratory in a refrigerated condition and stored in a refrigerator until desalination.

Desalted DSW and SSW being tested

The newest approach to desalination employs membrane systems, which include reverse osmosis and electrodialysis systems. We used an electrodialysis system to remove mainly Na^+ from DSW and SSW, because reverse osmosis system removes almost all of ions from the water. After analysis of key parameters such as nitrate, phosphate, silicate, and minerals, the dDSW and dSSW were kept in a deep freezer (–80°C) until use. After dissolution in a refrigerator, the water was diluted with purified water (Millipore Elix 5, Japan Millipore Corporation, Tokyo) to 3 concentrations by 5- (20%), 10- (10%), and 15- (6.7%) fold dilutions. Purified water was used as the control. Diluted dDSW, dSSW and purified water were administered orally to female BALB/c mice ad libitum for 12 weeks.

Animal treatment

A total of 110 female BALB/c mice (6 weeks old) were purchased from Charles River Japan Inc. (Yokohama). Female mice were used in this study because male BALB/c mice bite at each other when they are housed in the same cage. After pre-breeding for 1 week, the mice were randomly divided into 7 groups of 5 mice each and provided with 6.7%, 10%, or 20% dDSW or dSSW, or purified water as a control. The mice

were housed in 22 cages. Lighting was maintained on a 12-hour light/dark cycle, and the room temperature was kept at 23±2°C. Water intake was measured twice a week, while consumption of food (CE-2, CLEA Japan Inc., Tokyo), and body weight were measured once per week. The water intake, food consumption and body weight were averaged each week. To obtain a sample for hematologic tests, 5 mice were anesthetized with diethyl ether and were humanely killed before administration. The sample was collected from each mouse by an intracardiac puncture and was transferred into an anticoagulant tube containing EDTA-2Na. The blood collection for hematologic tests was carried out at the end of 1, 4, and 12 weeks.

Only one blood collection for blood chemical tests was carried out, 12 weeks after the administration. Blood was transferred into a serum separation tube and was centrifuged at 3000 rpm for 5 minutes. After the separation, the serum was stored at –80°C until determination.

This study was carried out under the approval of the Ethical Committee on the Treatment of Experimental Animals at Niigata University.

Hematologic and blood chemical tests

An automated hematology analyzer (K-4500, Sysmex Corporation, Kobe) was used for the determination of white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), Hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Differential WBC was performed using microscopic examination of blood smears stained by a modification of the May-Grunwald-Giemsa's stain (Dacie and Lewis. 1984). At least 200 cells were counted to produce each differential.

An automatic analyzer (Clinical Analyzer 7170, Hitachi High-Technologies Corp., Tokyo) was used to measure the following serum chemistries: total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), triglyceride (TG), and glucose (GLU). The methods used were as follows: TC, TG, and GLU by enzyme assay; and HDLC by direct enzyme method. Reagents and calibrators were purchased from International Reagents Corp., (Kobe) for TC and TG; from Denka Seiken Co., Ltd. (Tokyo) for HDLC; and from Roche Diagnostics K.K. (Tokyo) for GLU.

Statistical analysis

Data obtained from the diluted dDSW, dSSW, and control groups were recorded using Statistical Analysis System software (SAS Institute Inc., Cary, NC, USA). All data were compared by one-way ANOVA by Dunnett's test. A result was considered significant when the p-value was less than 0.05.

Results

Table 1 shows the values of key parameters in DSW and SSW before and after desalination. After desalination, the concentrations of potassium, calcium, magnesium, sodium, and chloride ions in DSW and SSW decreased 3.4-63.9% and 4.6-69.0%, respectively. Nitrate-nitrogen concentration decreased nearly 99% after the desalination, while no perceptible changes were found in the concentrations of phosphate-phosphorus and silicate-silicon.

Table 2 shows the effects of diluted dDSW and dSSW on water intake, food consumption, and weight gain. As expected, none of the groups of mice showed any clear abnormal growth or behavior; neither did any show signs of illness nor was there a single case of death during the 12-weeks period. Mean water intake and food consumption were averaged for each group every week. Weight gain was calculated from the values between before and 12 weeks after the administration. Mean water intake was significantly lower in the 20% dDSW, and in all the dSSW groups than in the control group. Significant differences for food consumption were also obtained in the 6.7% and 10% SSW groups. Weight gain did not significantly differ in the 7 water groups.

Table 3 shows the effects of diluted dDSW

TABLE 1. *Values of key parameters in deep seawater and surface seawater before and after desalination*

Parameter	Deep seawater		Surface seawater	
	Before	After	Before	After
Nitrate (NO_3-N)	1.29	0.02 (1.5)	0.11	<0.02 -
Phosphate (PO_4-P)	0.16	0.22 (137.5)	0.0	0.0 -
Silicate (SiO_2-Si)	1.24	1.38 (111.6)	0.24	0.28 (116.6)
Potassium	392	22 (5.7)	392	30 (7.6)
Calcium	519	220 (42.4)	515	244 (47.4)
Magnesium	1420	907 (63.9)	1370	945 (69.0)
Sodium	10361	354 (3.4)	9872	455 (4.6)
Chloride	19400	2697 (13.9)	18800	3020 (16.1)
Copper	0.0052	NM	0.0004	NM
Zinc	0.0297	NM	0.0010	NM
Manganese	0.0007	NM	0.0004	NM
Selenium	<0.01	NM	<0.01	NM

Values are expressed as mg/liter.
The value shown in the parenthesis is the percentage of the value before the desalination.
The values below the detection limit of the method are shown as <0.02, <0.01.
NM: Not measured.

TABLE 2. *Effects of desalted deep seawater and surface seawater diluted with purified water on water intake, food consumption, and weight gain*

Parameter	Desalted deep seawater			Desalted surface seawater			Control
	6.7%	10%	20%	6.7%	10%	20%	
Water intake (ml)	29.3±1.4	29.5±2.1	27.8±1.1**	26.7±1.0**	27.2±1.3**	27.9±1.5**	29.6±1.4
Food consumption (g)	19.7±0.8	20.2±0.9	19.7±0.8	18.8±0.6**	19.0±0.8**	19.8±1.0	19.8±1.1
Weight gain (g)	6.5±1.0	6.2±0.6	6.5±0.5	6.4±0.2	5.3±0.7	5.3±0.7	6.3±0.9

Values are means±S.D. of 5 mice.
Desalted deep seawater and surface seawater were diluted with purified water.
** Significantly different from the control group at $p<0.01$.

and dSSW on hematologic values at the end of 1, 4, and 12 weeks administration. One-way ANOVA was used to compare the mean values among the 7 water groups because significant differences between before and after the administration were found in the hematologic parameters. At the end of 12 weeks administration, mean RBC were significantly higher in the 6.7% dDSW and 20% dSSW groups than in the control group. Mean Hb and Ht levels were significantly higher in the 20% dSSW group than in the control group at that time. On the other hand, mean WBC was found to be significantly higher in the dSSW group than in the control group. At the end of 4 and 12 weeks administration, mean WBC and lymphocytes showed significant higher levels in the 10% dSSW group. Increased WBC in the dSSW group was associated with an increase of lymphocytes. We found no significant differences in MCV, MCH, MCHC or differential WBC except for lymphocyte.

Table 4 shows the effects of diluted dDSW and dSSW on blood chemical values. Mean TG level was significantly higher in the 6.7% dSSW

TABLE 3. *Effects of desalted deep seawater and surface seawater diluted with purified water on hematologic values*

Parameter	Desalted deep seawater			Desalted surface seawater			Control
	6.7%	10%	20%	6.7%	10%	20%	
Red blood cell count (×10¹²/liter)							
1 week	10.13±0.27	9.71±0.37	9.69±0.31	9.91±0.53	9.93±0.31	9.97±0.23	9.79±0.40
4 week	9.56±0.23	9.55±0.32	9.14±1.14	9.68±0.41	9.39±0.34	9.47±0.29	9.56±0.35
12 week	9.94±0.48*	9.47±0.61	9.47±0.47	9.35±0.22	9.59±0.34	10.08±0.29**	9.10±0.37
Hemoglobin (g/liter)							
1 week	1.69±0.05	1.63±0.06	1.60±0.04	1.62±0.10	1.61±0.04	1.67±0.06	1.61±0.06
4 week	1.60±0.04	1.60±0.06	1.62±0.02	1.59±0.13	1.59±0.06	1.61±0.05	1.63±0.05
12 week	1.59±0.07	1.56±0.11	1.54±0.05	1.56±0.04	1.58±0.07	1.67±0.05**	1.49±0.06
Hematocrit (%)							
1 week	56.7±2.3	54.4±1.7	53.1±1.8	53.6±3.1	54.7±1.1	55.6±2.3	54.3±3.0
4 week	52.4±1.4	52.7±2.2	53.0±0.9	53.2±2.1	52.4±1.8	52.3±1.8	53.1±2.0
12 week	53.8±2.8	51.9±3.6	51.6±2.7	50.5±1.5	51.6±2.1	54.9±1.8*	50.0±2.7
While blood cell count (×10⁹/liter)							
1 week	3.68±0.95	4.10±0.64	2.76±1.02	3.10±1.53	4.05±1.43	3.68±1.11	3.32±1.30
4 week	2.34±0.64	3.02±1.23	3.80±0.97	3.90±1.34	4.32±1.02*	3.64±1.06	2.26±1.09
12 week	4.68±1.61	4.48±0.71	2.90±0.56	3.86±1.12	4.92±1.57*	3.64±1.15	2.62±0.87
Neutrophil count (×10⁹/liter)							
1 week	0.86±0.19	0.74±0.22	0.54±0.29	0.74±0.61	0.88±0.30	0.95±0.35	0.72±0.38
4 week	0.54±0.25	0.70±0.27	0.92±0.47	0.99±0.52	0.80±0.42	0.61±0.19	0.59±0.27
12 week	1.46±0.76	1.72±0.36	1.07±0.42	0.94±0.35	1.77±0.81	1.17±0.34	1.22±0.48
Lymphocyte count (×10⁹/liter)							
1 week	2.76±0.85	3.33±0.56	2.19±0.79	2.33±1.06	3.09±1.32	2.67±1.37	2.53±0.93
4 week	1.75±0.49	2.24±1.05	2.86±0.64	2.87±1.06	3.46±0.65*	2.99±1.14	1.66±0.89
12 week	3.13±1.22*	2.65±0.50	1.77±0.43	2.79±1.15	3.00±0.73*	2.42±0.85	1.34±0.49

Values measured 1, 4 and 12 weeks after the administration of the waters are means±S.D. of 5 mice.
Deep seawater and surface seawater were diluted with purified water.
* Significantly different from the purified water group at $p<0.05$.
** Significantly different from the purified water group at $p<0.01$.

group than in the control group. In addition, TG in the dSSW group showed a tendency to decrease with the increases in the concentration of dSSW. There were no significant variations in TC, HDLC, and GLU levels among the 7 water groups.

DISCUSSION

This study demonstrated the safety and reliability for the utilization of diluted dDSW in the field of food processing and fresh water production. We need a study in human subjects in order to reveal the health effects of diluted dDSW on human beings. A previous study using commercially manufactured dDSW (Amami no Mizu Hardness 1000, Ako Kasei Co. Ltd., Hyogo) has suggested that the water may improve skin symptoms and mineral imbalances, and decrease IgE production and IgE-inducing cytokines, interleukin-4, interleukin-13, and interleukin-18 (Kimata

TABLE 4. *Effects of desalted deep seawater and surface seawater diluted with purified water on blood chemical values*

Parameter	Desalted deep seawater			Desalted surface seawater			Control
	6.7%	10%	20%	6.7%	10%	20%	
Total cholesterol	2.20±0.16	2.10±0.23	2.12±0.16	2.25±0.16	2.07±0.07	2.35±0.18	2.16±0.18
HDL-cholesterol	1.04±0.08	0.94±0.14	0.99±0.09	1.00±0.03	0.95±0.05	1.08±0.08	0.96±0.10
Triglyceride	1.06±0.29	1.24±0.15	1.14±0.16	1.64±0.68**	1.35±0.26	1.21±0.30	0.86±0.31
Glucose	10.61±2.25	9.63±0.95	11.45±2.32	12.07±2.09	9.94±1.11	11.20±1.96	11.68±3.48

Values are means±S.D. (mmol/liter) of 5 mice.
Deep seawater and surface seawater were diluted with purified water.
** Significantly different from the purified water group at $p<0.01$.

et al. 2002). Kimata et al. (2002) conducted the trial to study the effects of ready-made drinks on patients with atopic eczema/dermatitis syndrome by administering 500 ml per day of the drink for 1 year. In general, however, keeping the subjects' physical conditions constant over an extended time is not easy. Hematologic and blood chemical values, or human hormones vary according to daily physical conditions. We found a significant difference in leukocytes before the experiment, in spite of the subjects' normal life for three days demanded (Tsuchiya et al. 2003a). For these reasons, we conducted this study using mice to get fundamental data first.

The reasons why 3 concentrations (6.7%, 10%, and 20%) of dDSW and dSSW were taken into consideration are as follows. First, our previous study using mice demonstrated that serum IgG and GLU concentrations in the 12% DSW group diluted with purified water showed significant increases compared with those in the control group (purified water), while no significant differences were found in the 1.2% DSW group. Therefore, the 5- (20%) and 10- (10%) fold dilutions of dDSW and dSSW were selected as the concentrations to be studied. Secondly, water hardness of dDSW and dSSW were about 4500 mg/liter and 4200 mg/liter, respectively, when the value was calculated as $CaCO_3$ (Formula: Calcium concentration×2.5+Magnesium concentration×4). Because these are very high measures of water hardness, the 15-fold dilution (6.7%) was calculated based on approximately 200 mg/liter, which is the tap water hardness in Okinawa, the highest in Japan (Nakamura et al. 1988).

No acute or subacute toxicity of diluted dDSW was found in mice because the mice showed no signs of illness or a single case of death for 12 weeks after the administration. In the 10% and 20% dSSW groups, however, weight gain during the 12-week period was slightly lower than in the control group. This finding shows the possibility that dSSW may contain some substances which limit water intake in mice.

In order to compare the serial changes of hematologic values in the 7 water groups, measurements were performed before and after 1, 4, and 12 weeks of administration. Although RBC, Hb, and Ht levels showed no significant differences until 4 week of administration among the 7 water groups, those in the 20% dSSW group were significantly higher at the end of 12 weeks. In addition, RBC showed a significantly higher level in the 6.7% dDSW group at that time. Nevertheless, significant differences between 1 and 12 weeks after the administration were obtained in the control group. Therefore, we considered that these significant differences were caused by the decrease in the control group at the end of 12 weeks. Increased WBC and lymphocytes were found in the 10% dSSW group. Although the present study did not identify the existence of an outside substance, some microorganism may have caused the elevated lymphocyte.

In the blood chemical values, TG level was significantly higher in the 6.7% dSSW group, while no significant differences in TC, HDLC, and GLU were found among the 7 water groups. Inverse association between magnesium intake and plasma TG level has been previously reported (Altura et al. 1990; Ouchi et al. 1990; Sherer et al. 1999; Ravn et al. 2001; Cohen et al. 2002). As shown in Table 1, dDSW and dSSW are rich in calcium and magnesium. Magnesium in dDSW and dSSW is considered a possible reason for the decreasing TG level. Despite this evidence, the mean TG value was significantly higher in the 6.7% dSSW group 12 weeks after the administration than in the control group. Furthermore, no significant difference between the dDSW and control groups was found in the TG level. We could not define the cause of these differences in the present study. However, TG levels in the dSSW group showed a tendency to decrease with the increase in the concentration of dSSW. Magnesium in dSSW may have a considerable influence on serum TG level in mice, possibly explaining, at least partially, the decrease in serum TG level in this experiment; it is contained in dDSW as much as in dSSW. Since each type of water can potentially decrease the TG level, further research is necessary to determine why dDSW did not have this effect. Experiments using morbid state model animals may be helpful in establishing the relation between serum TG level and magnesium concentration in DSW or SSW.

On the other hand, inverse association between calcium or magnesium intake and plasma TC level has been previously reported (Yamaguchi et al. 1994; Ravn et al. 2001; Cohen et al. 2002, Nebrand et al. 2003). A previous animal experiment using DSW and mice has suggested that DSW had an effect of decreasing serum TC level (Suzuki 2000). This study was performed by using DSW collected from the Pacific Ocean, and male 14 weeks old Crj:CD-1 mice. Suzuki (2002) pointed to the possible role played by an abundance of silicate-silicon in DSW. However, a previous animal experiment using rats reported that silicon had the effects of decreasing of serum TG and low-density lipoprotein cholesterol, while no significant differences in serum TC and phospholipid were obtained (Najda et al. 1991). Our previous study using DSW collected from the Sea of Japan and BALB/c mice showed no significant difference in TC level. No significant difference in our studies might be due to the type, gender, and age of mice, or DSW used, or both. Otherwise, the decreased TC level may be caused by the elevated calcium and magnesium contents in DSW and SSW.

In the present study, no increase of mean serum GLU level was obtained in the dDSW groups, while our previous study demonstrated that the value in the 12% DSW group was significantly higher than that in the control group. Previous studies have reported on the relation between nitrate in drinking water and diabetes mellitus (Kostraba et al. 1992; Virtanen et al. 1994; Parslow et al. 1997). In the present study, nitrate-nitrogen concentration decreased nearly 99% after the desalination. This may be one of the reasons why no significant differences in GLU values were found between the dDSW and control groups.

In conclusion, our results show no evidence of acute or subacute effects of diluted dDSW. Effects of diluted dDSW on hematologic and blood chemical values in mice are thought to be similar to those of purified water. Our findings suggest that dDSW is as safe as purified water for drinking water.

Acknowledgments

This study was supported by a Grant-in-Aid from Niigata Prefecture as a part of the Deep Seawater Utilization Technology Research and Development Program in 2003.

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