Subacute Effects of Deep-Sea Water from the Japan Sea on Blood Examination Values in Mice

Yasuo TSUCHIYA*¹, Kazutoshi NAKAMURA*¹, Hiroho SEKIKAWA*², Hiroki KAWAMURA*²,
Kunio MIYANISHI*³, Teiji ISHIZU*¹ and Masaharu YAMAMOTO*¹

*¹Division of Social and Environmental Medicine, Department of Community Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata

*²Division of Immunology and Medical Zoology, Department of Infectious Disease Control and International Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata

*³Department of Public Health, Niigata Women's College, Niigata

Abstract

Objectives: To clarify the characteristics of deep-sea water (DSW), we investigated the hematological, immunological and biochemical effects of DSW, specifically the so-called Japan Sea Proper Water (JSPW), samples of which we collected from the Japan Sea at a depth of about 300 meters.

Methods: Five groups of five mice each were orally administered, *ad libitum* for 12 weeks, one of the following: 1.2% DSW, 12% DSW, 1.2% surface-sea water (SSW), 12% SSW, or purified water (control).

Results: Among these groups, no significant differences were observed in the average reduction of water intake, food consumption or body weight. The mean corpuscular volume, however, was significantly lower ($p < 0.05$) in the 1.2% DSW group than in the control group. Moreover, serum immunoglobulin G and A values were significantly higher ($p < 0.05$) in the 12% DSW and the 12% SSW groups, respectively, compared with the control group. In addition, the serum glucose value in the 12% DSW group was significantly higher ($p < 0.05$) than in the control group.

Conclusions: The findings of the present study suggested the presence of some toxic components in DSW. Before a final answer is reached about whether DSW, and specifically JSPW, is bad for human health, the pathophysiology of findings such as the decreased mean corpuscular volume, the higher immunoglobulin G value and the higher glucose value should be investigated.

Key words: deep-sea water, Japan Sea Proper Water, subacute effect, blood examination, mice

Introduction

Deep-sea water (DSW) is usually defined as water that circulates at a depth of more than 200 meters. In recent years, DSW has been commercialized in several areas, including the Sea of Japan. The characteristics of DSW include high concentrations of several types of inorganic nutrient salts, low temperature stability and low levels of pathogenic microorganisms compared with surface-sea water (SSW) (1). For these reasons, DSW is attracting much attention in Japan as a new national resource. DSW has already been drawn from several places in Japan (e.g., Kochi, Toyama, Okinawa and Kanagawa). DSW utilization technology has been

developed for the fields of agriculture, fisheries, food processing, medicine and health, among others (1).

Since March 2001, we have been drawing DSW from the Sado offing of the Japan Sea off Niigata prefecture. This water is called Japan Sea Proper Water (JSPW). The total area of the Japan Sea is about 1/164 that of the Pacific Ocean (2). It appears that the abyssal circulation in the Japan Sea is different from that in other bodies, especially in the Pacific Ocean, and even the water's contents are different (3–5). Although many studies have focused on the characteristics of DSW in the Pacific Ocean, very few have reported on DSW in the Japan Sea. To utilize JSPW effectively, it is very important to identify the characteristics of this water from the standpoint of its usability and safety.

There are some studies about medical and health-related applications of DSW (1, 6). However, the mechanisms underlying these healthful attributes have not been clarified completely. As one method for revealing the characteristics of DSW scientifically, an acute or subacute toxicity test of DSW is required. For this reason, we investigated the hematological, immunological and biochemical effects of DSW on the blood examination values of mice

Received Feb. 1 2002/Accepted May 28 2002

Reprint requests to: Yasuo TSUCHIYA

Division of Social and Environmental Medicine, Department of Community Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8510, Japan

TEL: +81(25)227-2121, FAX: +81(25)227-0764

E-mail: troof@med.niigata-u.ac.jp

by administering DSW orally for 12 weeks.

Materials and Methods

Collection of DSW and SSW

DSW and SSW were collected from respective depths of about 300 meters and 5 meters in the Japan Sea (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, Japan). The collected water was sent immediately to our laboratory in refrigerated condition and was stored at -80°C until use. After dissolution in the refrigerator, the water was diluted with purified water (Millipore ELIX 5, Japan Millipore Corporation, Tokyo) to two concentrations, 1.2% and 12%. Suzuki et al. (7) identified 1.2% as the concentration that represented the average daily consumption of DSW in the Japanese diet, as calculated from the consumption of fermentation foods (e.g., bean paste, soy sauce) and fish cakes (e.g., fish paste, fish sausage) based on the National Nutrition Survey in 1995 and the production processes of these foods. Then, the concentration was converted to a per-mouse basis according to the daily food consumption and water intake of a mouse. This study was carried out also at the 12% concentration. Diluted DSW, SSW or the control (purified water) was administered orally to the mice *ad libitum* for 12 weeks.

Animal treatment

Twenty-five female BALB/c mice (6 weeks old) were purchased from Charles River Japan Inc. (Yokohama, Japan). 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 divided into five groups of five mice each and housed in five cages, one group per cage, for 12 weeks. Lighting was maintained on a 12-hour light/dark cycle, and the room temperature was kept at $23\pm 2^{\circ}\text{C}$. Water intake was measured twice a week, while consumption of food (CF-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. After the end of the 12-week administration, the mice were anesthetized with diethyl ether and were sacrificed. A blood sample was collected from each mouse by an intracardiac puncture. A 300 μl blood sample used for the hematological examination was transferred into an anticoagulant tube containing EDTA-2Na. The remaining blood sample was transferred into a serum separation tube for the immunological and biochemical examinations and was centrifuged at 3,000 rpm for 5 minutes. After the separation, the serum was stored at -80°C until determination.

The study was carried out under the approval of the Animal Experiment Committee of Niigata University (July 5, 2001).

Hematological, immunological and biochemical determinations

An automated hematology analyzer (K-4500, Sysmex Corporation, Kobe, Japan) was used for the determination of white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT) and mean corpuscular volume (MCV).

Serum concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were determined by the single radio immunodiffusion (SRID) method (The Binding Site Ltd., Birmingham, UK).

Serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL), low-density lipoprotein-cholesterol (LDL), triglyceride (TG), urea nitrogen (UN), glucose (GLU) and total protein (TP) were determined by an automatic analyzer (COBAS MILA, Roche Japan, Tokyo). The methods used were as follows: TC, TG, UN and GLU by enzyme assay; HDL and LDL by the direct enzyme method; and TP by the biuret reaction. All reagents and calibrators were purchased from Daiichi Pure Chemicals (Tokyo, Japan).

Statistical analysis

Data obtained from the DSW, SSW and control groups were recorded using Statistical Analysis System software (SAS Institute Inc., Cary, NC, USA). Dunnett's test was used to calculate p-values. A result was considered significant when the p-value was less than 0.05.

Results

As shown in Fig. 1, there were no significant differences between the five groups in water intake (top of figure) or food consumption (middle), even though both appeared slightly higher in the 12% DSW group than in the other groups. The average body weight (bottom) also did not differ significantly between the groups. The growth curves of all five groups showed similar fluctuations.

Table 1 shows the results of the hematological examinations. The 1.2% DSW group had significantly decreased MCV compared with the control group ($p<0.05$). There were no significant variations in WBC, RBC, Hb or PLT in the DSW and SSW

Fig. 1 Average reduction in water intake, food consumption and body weight. Control, closed triangle; 1.2% DSW, open circle; 12% DSW, closed circle; 1.2% SSW, open square; 12% SSW, closed square.

Table 1 Effects of deep-sea water and surface-sea water on hematological values^a

	WBC ($\times 10^3/\text{mm}^3$)	RBC ($\times 10^6/\text{mm}^3$)	Hb (g/dl)	PLT ($\times 10^3/\text{mm}^3$)	MCV (fl)
1.2% DSW	2.86 $\pm$ 0.87	10.42 $\pm$ 0.42	16.8 $\pm$ 0.4	913 $\pm$ 72	50.7 $\pm$ 0.7*
12% DSW	2.94 $\pm$ 1.59	10.10 $\pm$ 0.66	16.4 $\pm$ 0.8	985 $\pm$ 49	51.2 $\pm$ 0.3
1.2% SSW	2.84 $\pm$ 1.29	10.23 $\pm$ 0.35	16.7 $\pm$ 0.5	827 $\pm$ 165	51.7 $\pm$ 0.9
12% SSW	3.42 $\pm$ 1.39	10.11 $\pm$ 0.37	16.7 $\pm$ 0.8	958 $\pm$ 85	51.3 $\pm$ 0.8
Control	4.78 $\pm$ 1.59	9.87 $\pm$ 0.55	16.0 $\pm$ 0.7	874 $\pm$ 58	52.2 $\pm$ 1.1

^a Mean $\pm$ S.D. of five mice per group.* Significantly different from control, purified water, at $p < 0.05$.**Table 2** Effects of deep-sea water and surface-sea water on immunological values^a

	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
1.2% DSW	283 $\pm$ 13.1	178 $\pm$ 22.2	52 $\pm$ 1.7
12% DSW	421 $\pm$ 112.0*	202 $\pm$ 8.1	54 $\pm$ 1.6
1.2% SSW	264 $\pm$ 13.6	168 $\pm$ 6.3	52 $\pm$ 1.5
12% SSW	321 $\pm$ 41.6	237 $\pm$ 20.4*	52 $\pm$ 4.8
Control	276 $\pm$ 30.5	190 $\pm$ 18.2	52 $\pm$ 3.3

^a Mean $\pm$ S.D. of five mice per group.* Significantly different from control, purified water, at $p < 0.05$.

groups, compared with those of the control group.

Table 2 shows the serum IgG, IgA and IgM values determined by the SRID method. The serum IgG value in the 12% DSW group was significantly higher than that in the control group ($p < 0.05$). In serum IgA values, the 12% SSW group was significantly higher than the control group ($p < 0.05$). All five groups of mice had essentially similar serum IgM values.

Table 3 shows the biochemical values in the five groups. The 12% DSW group had significantly elevated GLU values compared with the control group ($p < 0.05$). Other biochemical values in the DSW and SSW groups were not found to be significantly different from those of the control group.

Discussion

Seawater accounts for 97.6% of Earth's water. Furthermore, seawater below a depth of 200 meters (DSW) accounts for about 95% of all seawater. The water surrounding Japan includes several masses, such as JSPW, North Pacific intermediate water and Pacific Deep Water. It is expected that the advanced utilization of these waters, either in the water itself or in microorganisms contained therein, can help solve such problems as environmental pollution and the needs for energy and food security. To promote these efforts, it is very important to first elucidate the characteristics of DSW. For example, Nomura et al. (6) used DSW to treat atopic dermatitis and obtained an effective rate of about 60%. However, the mechanisms underlying these healthful uses and the differences between DSW and SSW or saline solution were not

completely clarified. The roles that the various characteristics of DSW play in these findings need to be studied further.

JSPW, which flows from the Vladivostok coastal waters, is different from the North Atlantic Deep Water that flows from the Nordic seas (2–5). The components of JSPW may be different from those of other DSW. In fact, a few differences between the components of DSW collected in Kochi and those collected in Toyama were reported (8). There are also some well-known differences between the components of DSW and those of SSW (1, 7).

The committee of Japan Sea Proper Water Development and Research collected DSW and SSW experimentally from the Japan Sea in October, 2000 and again in March, 2001. The water samples were then analyzed to identify their components. No evident differences between DSW and SSW were found other than that SSW had higher levels of dissolved oxygen (DSW, 7.0 mg/l; SSW, 9.3 mg/l) while DSW had higher levels of phosphate-phosphorus (DSW, 1.8 $\mu\text{mol/l}$; SSW, 0.3 $\mu\text{mol/l}$), silicate-silicon (DSW, 48.4 $\mu\text{mol/l}$; SSW, 7.7 $\mu\text{mol/l}$) and nitrate-nitrogen (DSW, 25.5 $\mu\text{mol/l}$; SSW, 1.2 $\mu\text{mol/l}$) (9). Except for the water temperature (Niigata, 2.4°C; Kochi, 8.1–9.8°C), these characteristics of JSPW were similar to the results of DSW from the Pacific Ocean.

In the present study, we investigated the effects of JSPW on the blood examination values of mice in order to announce its characteristics and its differences from other DSWs. The salient finding in hematological values in the mice is that the 1.2% DSW group had lower MCV than the control group. There are no known studies about the relation between DSW administration to mice and the animals' MCV levels. In humans, and specifically infants, a relation between nitrate-nitrogen and nitrite-nitrogen intake was reported, as was the development of methemoglobinemia in infants (10–13). DSW contains higher concentrations of these salts than either SSW or purified water. The decrease in MCV may be induced by nitrate-nitrogen and nitrite-nitrogen, but there is no clear evidence to support this interference, because MCV in the 12% DSW group did not show a significant difference compared with that in the control group. Therefore, further studies are needed to clarify the effect of DSW on the decreased MCV.

In addition, there are several studies that reported about the relation between nitrate in drinking water and diabetes mellitus

Table 3 Effects of deep-sea water and surface-sea water on biochemical values^a

	TC (mg/dl)	HDL C (mg/dl)	LDLC (mg/dl)	TG (mg/dl)	TP (g/dl)	UN (mg/dl)	GLU (mg/dl)
1.2% DSW	69 $\pm$ 8.5	49 $\pm$ 0.8	6.2 $\pm$ 0.9	39 $\pm$ 6.2	5.1 $\pm$ 0.1	22 $\pm$ 1.0	193 $\pm$ 44
12% DSW	74 $\pm$ 5.2	48 $\pm$ 4.8	7.0 $\pm$ 0.4	82 $\pm$ 35.5	5.2 $\pm$ 0.4	24 $\pm$ 3.4	252 $\pm$ 21*
1.2% SSW	80 $\pm$ 5.2	50 $\pm$ 6.9	7.6 $\pm$ 1.4	73 $\pm$ 25.9	5.4 $\pm$ 0.3	20 $\pm$ 1.2	179 $\pm$ 26
12% SSW	80 $\pm$ 3.6	52 $\pm$ 1.5	6.8 $\pm$ 0.3	72 $\pm$ 37.1	5.5 $\pm$ 0.1	24 $\pm$ 1.2	181 $\pm$ 38
Control	75 $\pm$ 5.1	50 $\pm$ 7.2	7.3 $\pm$ 1.2	57 $\pm$ 8.6	5.0 $\pm$ 0.3	21 $\pm$ 1.2	183 $\pm$ 28

^a Mean $\pm$ S.D. of five mice per group.* Significantly different from control, purified water, at $p < 0.05$.

(14–16). Kostraba et al. (14) reported that low-level nitrate exposure through drinking water might play a role in the etiology of insulin-dependent diabetes mellitus, perhaps in generating free radicals. The mechanism was suggested to work in the following way: Nitrate changes into nitrite by the hydrogen ion that exists in saliva and in the alimentary canal. This nitrite generates nitrosamines after it reacts with proteins. Free radicals discharged from the nitrosamines attack the B cells in the pancreas. As a result, the generation of insulin is controlled, causing diabetes. The present study showed that the 12% DSW group had significantly higher GLU values than the control group. The concentration of nitrate in DSW was higher than in either the SSW or the purified water. The elevated serum GLU values may be caused by the long-term intake of high concentrations of DSW.

To our knowledge, the effects of DSW on serum immunoglobulin values in mice have not been investigated. The present findings showed that the serum IgG value in the 12% DSW group and the serum IgA value in the 12% SSW group were significantly higher than in the control group. One of the functions of IgG is similar to that of IgA. That is, both IgG and IgA act as neutralizing antibodies (17). Although the present study did not announce the existence of an identified substance (e.g., a microorganism, a mineral) that influences immunoglobulin values, it is suggested that the elevated IgG and IgA values may be due largely to differences in some components between DSW and SSW. It is possible that a useful substance for the infection defense is contained in DSW or SSW. These findings show that the long-term use of DSW or SSW stimulates the immune system when taken in a high concentration. Further studies that eliminate microorganisms from

the water are needed to confirm the immunological effects of DSW or SSW.

Previous studies suggested that silicate-silicon contained in DSW might decrease TC values in mouse plasma (8, 18). Those studies were carried out by using DSW from the Pacific Ocean and male mice (Crj: CD-1, 14 weeks old). However, the present results do not confirm those findings. In the present study, the TC values in the DSW group showed a slight decrease, especially in the 1.2% group, and the mechanism of the decreased TC value was not clear. This difference might be due to the type and gender of mouse and/or to the DSW used.

The present study suggested the presence of some toxic components in DSW, even though none of the groups of mice showed any clear abnormal growth or behavior; neither did any show signs of illness or a single case of death during the study period. Further careful study of the advantages or disadvantages of DSW are necessary before a final answer is reached regarding whether DSW is bad for human health. Problems that still need to be investigated include the confirmation of the pathophysiology of the decreased MCV, the higher IgG values and the higher GLU values.

Acknowledgements

This work was supported by a Grant-in-Aid from Niigata prefecture as part of the Deep Sea Water Utilization Technology Research and Development Program in 2001. We are grateful to Dr. N. Fujisawa and Mr. Y. Maeda for their invaluable advice on the animal treatment.

References

- (1) Sasaki T. Deep Sea Water. Tokyo: KK-Bestsellers, 2001. (in Japanese)
- (2) Senjyu T. Formation and circulation of the upper portion of the Japan Sea Proper Water as deduced from isopycnal analysis. *Kaiyo* 1994; 12: 794–801. (in Japanese)
- (3) Nitami H. On the deep and bottom waters in the Japan Sea. In “Researches in hydrography and oceanography”, ed. by Shoji, D., Hydrographic Department of Japan Maritime Safety Agency. Tokyo 1972; 151–201.
- (4) Gamo T, Horibe Y. Abyssal circulation in the Japan Sea. *J. Oceanogr. Soc. Japan* 1983; 39: 220–230.
- (5) Sudo H. A note on the Japan Sea Proper Water. *Prog. Oceanogr.* 1986; 17: 313–336.
- (6) Nomura I, Matsumoto K, Fujieda M, Morita H, Kurashige T, Tanabe S, Yamaguchi M. Treatment of atopic dermatitis using deep sea water. *Allergy no rinsyo* 1996; 16(6): 37–40. (in Japanese)
- (7) Suzuki H. Characteristics, utilization and functionality of deep-sea water. *Food Research* 2000; 7: 37–41. (in Japanese)
- (8) Ito M, Suzuki H. Characteristics and utilization of deep-sea water. *Food Style* 21 2000; 4(7): 60–63. (in Japanese)
- (9) The committee of Japan Sea Proper Water Development and Research. Toward the profit utilization of Sado deep-sea water, the 2nd meeting 2001. (in Japanese)
- (10) Ayebo A, Kross BC, Vlad M, Sinca A. Infant methemoglobinemia in the Transylvania region of Romania. *Int. J. Occup. Environ. Health* 1997; 3(1): 20–29.
- (11) Gelgerg KH, Church L, Casey G, London M, Roerig DS, Boyd J, Hill M. Nitrate levels in drinking water in rural New York State. *Environ. Res.* 1999; 80(1): 34–40.
- (12) Gupta SK, Gupta RC, Seth AK, Gupta AB, Bassin JK, Gupta A. Methaemoglobinaemia in areas with high nitrate concentration in drinking water. *Natl. Med. J. India* 2000; 13(2): 58–61.
- (13) Sanchez EJ, Benito FJ, Mintegui RS. Methemoglobinemia and consumption of vegetables in infants. *Pediatrics* 2001; 107(5): 1024–1028.
- (14) Kostraba JN, Gay EC, Rewers M, Hamman RF. Nitrate levels in community drinking waters and risk of IDDM. An ecological analysis. *Diabetes Care* 1992; 15(11): 1505–1508.
- (15) Virtanen SM, Jaakkola L, Ylonen K, Lounamaa R, Akerblom HK, Tuomilehto J. Nitrate and nitrite intake and the risk for type 1 diabetes in Finnish children. *Childhood Diabetes in Finland Study Group. Diabet. Med.* 1994; 11(7): 656–662.
- (16) Parslow RC, Mckinney PA, Law GR, Staines A, Williams R, Bodansky HJ. Incidence of childhood diabetes mellitus in Yorkshire, northern England, is associated with nitrate in drinking water: an ecological analysis. *Diabetologia* 1997; 40: 550–556.
- (17) Jeneway CA Jr., Travers P, Hunt S, Walport M. *IMMUNOBIOLOGY—The Immune System in Health and Disease*, Third Edition. London: Garland Publishing Inc. 1994.
- (18) Ito M, Suzuki H. Utilization of deep-sea water. *Kouryo* 2000; 206: 83–96. (in Japanese)