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Original Article

Effects of Hot Deep Seawater Bathing on the Immune Cell Distribution in Peripheral Blood from Healthy Young Men

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Abstract

Objectives: Deep seawater (DSW) utilization technology has been developed for the fields of medicine and health, among others. To clarify the health effects of DSW as compared with surface seawater (SSW) or tap water (TW), we investigated the changes of immune cell distribution of the peripheral blood, or subjective judgment scores, after hot water bathing.

Methods: Ten healthy young men were immersed for 10 min in DSW, SSW and TW heated to 42°C. Blood samples were collected before bathing, immediately after bathing and 60 min after bathing. Total and differential numbers of leucocytes and lymphocyte subsets (CD3, CD4, CD8, CD19, CD16, and CD56) were examined using an automated hematology analyzer and a flow cytometer, respectively. The subjective judgment scores were obtained by an oral comprehension test.

Results: Since the pre-bathing leukocyte count in the TW group was significantly different from those in the DSW and SSW groups, we excluded the findings of TW bathing from consideration. In hot DSW bathing, CD8-lymphocytes increased significantly immediately after bathing ($p<0.05$), in contrast to hot SSW bathing, in which no significant changes were detected in the lymphocyte subsets. Additionally, there were no significant changes between repeated measurements in the subjective judgment scores, though the score of thermal sensation in SSW bathing showed a significantly higher value immediately after bathing than before bathing ($p<0.01$).

Conclusions: Our findings suggest that increased CD8-lymphocytes in hot DSW bathing may improve human immune function as well as hot springs do, as compared with SSW bathing. Although hot DSW bathing may have the ability to change human immune cell distribution, well-designed studies are needed to clarify the health effects including not only DSW and SSW but also TW.

Key words: deep seawater, hot water bathing, immune response, leukocyte, lymphocyte subsets

Introduction

Deep seawater (DSW) is usually defined as water that circulates at a depth of more than 200 meters (1). In recent years, DSW has been commercialized in Japan and around in the world and developed for use in the fields of agriculture, fisheries, food processing, medicine and health. A few studies exist concerning the medical and health-related applications of DSW (2, 3). Recently, the use of DSW in thalassotherapy has begun in Japan.

The Japanese make it a practice to bathe daily, and prefer hot water baths of 42°C or more to tepid water baths. In general, hot springs bathing is useful for maintaining hygiene, improving blood circulation and mitigating muscular fatigue and pain because of the water's warm temperature, pressure and buoyancy effect (4). In addition, it has been discovered that the salt in hot spring water has the effect of raising body temperature longer (4). Moreover, balneotherapy uses salt water for immunological effects; studies regarding the effects of balneotherapy in the treatment of some diseases at the Dead Sea have been published (5–8).

To clarify the efficacy of DSW to humans, we investigated

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changes in immune cell distribution in the peripheral blood before bathing, after bathing for 10 min in a water temperature of 42°C, and 60 min after bathing. The results for DSW were compared with those in surface seawater (SSW) and tap water (TW) bathing.

Subjects and Methods

Subjects

Subjects used in this study were 10 healthy male students with a mean age of 20.2 years (S.D., 3.3), mean height of 168.6 cm (S.D., 4.5) and mean weight of 60.7 kg (S.D., 3.1), who agreed to participate in this study. All subjects provided written informed consent immediately after they were informed of the purpose of this study and the potential risks involved, such as fluctuation of blood pressure after bathing, and that blood collection would occur three times a day. They were asked to take their last meal at least 12 hr before participating in this study. The Ethics Committee at Niigata University School of Medicine approved our methods.

Collection of DSW and SSW

DSW and SSW were collected from respective depths of about 300 meters and 10 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). Water was collected in plastic containers and sent immediately to our laboratory. The water was used within 5 days.

Experimental procedure

This study was performed using three kinds of water and two bathtubs for three consecutive days; the experiment assigned between 8 a.m. and 12 a.m. was repeated five times a day in July 2002. After two different types of water were poured into the two bathtubs, two subjects bathed in numeric order. The subjects were not told which type of water was used and were tested at the same time for three consecutive days to avoid the influence of physical conditions or circadian rhythms of leukocytes (9).

The experimental procedure was as follows: two experimental bathtubs (1.0×0.6×0.5 m depth) made of fiber-reinforced plastic were set up in a prefabricated bathroom (2.1×5.3×2.3 m height), where the temperature was maintained at 32±1°C and the relative humidity was kept at 70±1% during the experiment.

Two subjects entered a prefabricated waiting room (2.1×5.3×2.3 m height), where the temperature was maintained at 27±2°C and the relative humidity was kept at 56±2%. Here, subjects rested for 30 min before the start of the experiment. The first blood sample (2 ml) was collected from the median cubital vein of each subject and was transferred into an anti-coagulation tube containing EDTA-2Na. Thermal sensation, degree of fatigue, and comfort level were recorded at that time. These subjective judgments were evaluated as follows: thermal sensation, 1: cold, 2: cool, 3: slightly cool, 4: neutral, 5: slightly warm, 6: warm, 7: hot, 8: very hot; degree of fatigue, 1: not tired, 2: hardly tired, 3: slightly tired, 4: tired, 5: very tired; comfort level, 1: very comfortable, 2: comfortable, 3: normal, 4: slightly uncomfortable, 5: uncomfortable.

Water was poured into the bathtub. The water temperature was set at 42°C at the start of the experiment and was regulated by a temperature control device throughout the experiment. The subject moved from the waiting room to the bathroom and immersed himself in the bathtub with his legs extending forward. The surface of the water in the bathtub reached the level of the diaphragm. The bathing continued for 10 min. After bathing for 10 min, the subject left the bathtub and moved to a prefabricated resting room (2.1×5.3×2.3 m height), where the temperature (26±1°C) and the relative humidity (55±1%) were controlled, the latter by an air conditioner. The subject rested immediately on a reclining chair, at which time a second blood sample (2 ml) was collected. Thermal sensation, degree of fatigue, and comfort level were recorded at that time. After drying himself thoroughly with a towel, the subject moved to the waiting room and dressed, then rested and relaxed there until the third blood collection (2 ml), performed 60 min after bathing. At this point, each subject was again asked to report his subjective judgments.

Examination of peripheral blood

An automated hematology analyzer (SF-3000, Sysmex Corporation, Kobe, Japan) was used for the examination of total and differential leukocytes. The direct immunofluorescence technique was used for the deviation of lymphocyte subsets. Blood samples were stained with the following monoclonal antibodies directly labeled either with fluorescein isothiocyanate (FITC) and phycoerythrin (PE) or PerCP: CD4/CD8 (Simultest, Becton Dickinson Immunocytometry Systems, CA, USA) for the detection of CD4- and CD8-lymphocytes, and CD3/CD16+ CD56/CD19 (Simultest) for the detection of T-, natural killer (NK)- and B-cells. Flow cytometric analysis of 10,000 cells was performed with a FACScan (BD Biosciences, CA, USA) after performing a fine electrical adjustment of the system by the control (CaliBRITE, Becton Dickinson Immunocytometry Systems, CA, USA). Data analysis was performed with CELL Quest (BD Biosciences).

Statistical analysis

Statistical analyses were performed using Statistical Analysis System software (SAS Institute Inc., Cary, NC, USA). All data were expressed as mean±standard deviation. To compare mean, absolute and relative values between the three experimental conditions, analysis of variance (ANOVA) was used. We used Scheffe's test (the GLM procedure) to compare effects of hot water bathing for the total and differential leukocytes, lymphocyte subsets, and subjective judgment scores from before, immediately after and 60 min after bathing. A result was considered significant when the p-value was less than 0.05.

Results

Total and differential leukocytes

Table 1 shows the bathing-induced changes in the total and differential leukocytes. First of all, leukocytes before bathing among the three kinds of water were compared using one-way ANOVA. Although no significant difference between the values

Table 1 Hot water bathing-induced changes in total and differential leukocytes

	Before bathing	After bathing	60 min after bathing
DSW			
Leukocytes	4.99±0.67	5.32±0.84	5.19±0.81
Neutrophils	2.88±0.59	3.06±0.65	3.08±0.74
Lymphocytes	1.63±0.24	1.80±0.29	1.69±0.34
Monocytes	0.31±0.05	0.30±0.04	0.26±0.03*,†
Eosinocytes	0.14±0.09	0.13±0.09	0.13±0.09
Basophiles	0.03±0.01	0.03±0.01	0.03±0.01
SSW			
Leukocytes	5.44±0.77	5.67±1.01	5.46±1.13
Neutrophils	3.08±0.55	3.24±0.64	3.27±0.95
Lymphocytes	1.88±0.44	1.97±0.52	1.75±0.28
Monocytes	0.31±0.04	0.31±0.06	0.29±0.08
Eosinocytes	0.15±0.10	0.12±0.08*	0.13±0.09*
Basophiles	0.03±0.01	0.03±0.01	0.03±0.01
TW			
Leukocytes	6.29±1.36	6.40±1.49	5.80±1.09†
Neutrophils	3.85±1.24	3.96±1.27	3.62±0.96
Lymphocytes	2.02±0.63	2.00±0.56	1.58±0.33*,†
Monocytes	0.27±0.09	0.31±0.09	0.30±0.06
Eosinocytes	0.12±0.09	0.11±0.10	0.10±0.09
Basophiles	0.03±0.01	0.03±0.01	0.03±0.01

DSW: deep seawater, SSW: surface seawater, TW: tap water.
Values are mean±S.D. (10^9/l) of 10 subjects.
Significant differences compared with the value before bathing are indicated as * $p<0.05$.
Significant differences compared with the value after bathing are indicated as † $p<0.05$.

before DSW and SSW bathing was found, the value before TW bathing was significantly higher than those before DSW ($p<0.01$) and SSW ($p<0.05$) bathing. Since significant differences in leukocytes before bathing among the three water groups were found, a comparison between only DSW and SSW was performed. In DSW bathing, leukocytes showed no significant change immediately after and 60 min after bathing compared with before bathing. No significant changes are found in neutrophils, lymphocytes, eosinocyte and basophiles. Monocytes, however, were significantly lower at 60 min after bathing than before ($p<0.05$) or after bathing ($p<0.05$). The rates of decrease were approximately 15%. In SSW bathing, leukocytes, neutrophils, lymphocytes and basophiles showed changes almost identical to DSW. Eosinocyte levels were significantly lower after bathing ($p<0.05$) and 60 min after bathing ($p<0.05$) than before bathing. There was no significant change in monocytes, which showed significant decreases 60 min after bathing in the DSW group.

Lymphocyte subsets

Table 2 shows the bathing-induced changes in the lymphocyte subsets. In DSW bathing, CD8-lymphocytes increased significantly after bathing ($p<0.05$). Subsequently, they decreased to the pre-experimental value at 60 min after bathing, while no significant change was found. In SSW bathing, there were no significant changes in the lymphocyte subsets.

Table 2 Hot water bathing-induced changes in lymphocyte subsets

	Before bathing	After bathing	60 min after bathing
DSW			
CD3+	1.06±0.22	1.13±0.25	1.07±0.27
CD4+	0.56±0.24	0.61±0.27	0.60±0.27
CD8+	0.52±0.15	0.62±0.14*	0.55±0.16
CD19+	0.26±0.08	0.28±0.07	0.28±0.06
CD16+56	0.19±0.06	0.26±0.12	0.22±0.11
SSW			
CD3+	1.18±0.36	1.26±0.40	1.09±0.26
CD4+	0.63±0.26	0.69±0.25	0.61±0.26
CD8+	0.63±0.21	0.63±0.26	0.56±0.16
CD19+	0.28±0.06	0.27±0.05	0.30±0.07
CD16+56	0.24±0.10	0.24±0.14	0.21±0.09
TW			
CD3+	1.20±0.43	1.21±0.38	1.02±0.27
CD4+	0.65±0.31	0.66±0.30	0.59±0.26
CD8+	0.70±0.30	0.70±0.29	0.46±0.17**,††
CD19+	0.28±0.11	0.29±0.08	0.23±0.05†
CD16+56	0.37±0.17	0.33±0.17	0.20±0.10*

DSW: deep seawater, SSW: surface seawater, TW: tap water.
Values are mean±S.D. (10^9/l) of 10 subjects.
Significant differences compared with the value before bathing are indicated as * $p<0.05$, ** $p<0.01$.
Significant differences compared with the value after bathing are indicated as † $p<0.05$, †† $p<0.01$.

Table 3 Changes in subjective judgment scores

	Before bathing	After bathing	60 min after bathing
Thermal sensation			
DSW	3.9±0.83	4.8±1.83	3.6±0.92
SSW	3.3±0.78	4.8±1.08**	4.1±0.70
TW	4.8±1.17	6.4±1.20*	4.7±0.90††
Degree of fatigue			
DSW	2.1±0.70	2.5±0.50	2.1±0.70
SSW	2.4±0.66	2.6±0.66	2.3±0.64
TW	2.9±0.30	3.0±0.77	2.7±0.64
Comfort level			
DSW	3.0	2.5±0.67	2.7±0.64
SSW	2.8±0.40	2.7±0.46	2.4±0.66
TW	3.2±0.60	2.8±0.87	2.7±0.46

DSW: deep seawater, SSW: surface seawater, TW: tap water.
Values are mean±S.D. of 10 subjects.
Significant differences compared with the value before bathing are indicated as * $p<0.05$, ** $p<0.01$.
Significant differences compared with the value after bathing are indicated as †† $p<0.01$.

Subjective judgments

Table 3 shows the changes in the subjective judgment scores. No significant differences in the pre-bathing value of each score between DSW and SSW bathing were found. In DSW bathing, there were no significant changes in mean scores of thermal sensation, degree of fatigue, and comfort level between repeated measurements. In SSW bathing, however, the mean score (4.8) of thermal sensation after bathing was significantly higher than before bathing ($p<0.01$), though the value was similar to that in DSW bathing. There were no significant

changes in the mean scores of degree of fatigue and comfort level between repeated measurements.

Discussion

The utilization of DSW for health promotion has been marketed by various industries in Japan as well as around the world. Since taking a bath everyday is a general practice among most Japanese people, we evaluated the health effects of bathing in DSW from the viewpoint of immunology in comparison with those in SSW and TW bathing.

In spite of our experimental purposes mentioned above, we had to exclude the findings of TW bathing, since the pre-bathing leukocyte count in the TW group was significantly different from those in the DSW and SSW groups. We therefore make a comment on the health effects of DSW as compared with those of SSW.

In DSW bathing, mean monocytes showed a significant decrease during the 60-min resting period, while no significant difference between pre-bathing and post-bathing levels was observed. This change was not similar to the stress-induced change in rats (10). Previous study has demonstrated that B-cell, NK-cell and monocyte numbers in rats showed a greater stress-induced decrease largely due to adrenal hormones (11). Although we could not find the major factors causing this change 60 min after bathing, hormones might be involved in monocyte levels. Thus, hot DSW bathing-induced changes in immune cell distribution may comparable to stress or exercise-induced changes in rats.

Our data also indicated an increase of CD8-lymphocytes after DSW bathing; moreover, the value was maintained during the 60-min resting period. In a recent study by Watanabe et al. (12), leukocytes and lymphocytes with NK- and CD8-cells increased significantly after bathing and returned to the pre-experimental values 60 min after bathing. The study was performed on patients with rheumatoid arthritis who immersed in hot springs at 42°C for 5 min. In addition, it is well known that patients with rheumatoid arthritis show a decrease of CD8-lymphocytes. Thus, the increase of CD8-lymphocytes after DSW bathing may have a role in the treatments of rheumatoid arthritis as well as hot springs do. However, mean CD8-lymphocytes before DSW and SSW bathing were 0.52 and 0.63×10^9/l, respectively, though no significant difference was found. The small quantity of pre-experimental values between the two waters may explain the significant difference in CD8-lymphocyte. Otherwise, the hot DSW bathing-induced change in CD8-lymphocyte distribution may have been influenced by components that appear at higher levels in DSW than in SSW, such as nitrate-nitrogen, phosphate-phosphorus and silicate-silicon. These results suggest that hot DSW bathing may have the ability to change human immune function, while SSW bathing showed no such effect.

Regarding thermal sensation, the mean score for DSW bathing showed no significant changes between repeated measurements, while a significant increase of the score after bathing in SSW was obtained compared with before bathing. Both DSW and SSW contain approximately 3.5% salt (13). After DSW and SSW bathing, this salt remains on the skin and can prevent evaporation of sweat, thereby keeping the body temperature warmer. However, people generally have a sticky feeling after bathing in the ocean. Hot SSW bathing also leaves one with a sticky feeling, while DSW bathing does not. DSW has higher levels of nitrate-nitrogen, phosphate-phosphorus and silicate-silicon than SSW or TW (13). Moreover, it has a good balance of minerals. Being mineral-rich, with nitrate-nitrogen, phosphate-phosphorus and especially silicate-silicon might be one of the reasons that no significant difference in thermal sensation was found in DSW bathing compared with SSW bathing.

Although this was the first study to examine the association between hot DSW bathing and its induced changes in the immune cell distribution of peripheral blood, to our knowledge, this study had a number of limitations. In spite of the subjects' normal life for the three consecutive days demanded, significant differences in leucocytes and subjective judgment scores before bathing between TW bathing and DSW bathing or SSW bathing were obtained. For this reason, we had no other choice but to eliminate the results obtained in TW bathing from our discussion about the health effects of the three kinds of water.

One of the reasons for the differences is that keeping the subjects' physical conditions constant for three days was very difficult, because our study was conducted in the midsummer heat. In addition, the experiment was performed for three consecutive days. It is possible that bathing for three consecutive days influences the changes of the immune cell distribution on the second and third days. If similar studies using the three kinds of water are conducted in a temperate climate almost at the same time, researchers may eventually obtain more apparent results. Thus, further studies are needed to support our findings.

Our next trial is to clarify the health effects of tepid water bathing including not only DSW and SSW but also TW. Two previous studies (14, 15) suggest that tepid water bathing is more effective for fatigue and physiological indexes than hot water bathing. An experiment in tepid water, keeping the subjects' physical conditions constant before participating in the study, would help to confirm more clearly the efficacy of DSW.

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