Difference between Deep Seawater and Surface Seawater in the Preventive Effect of Atherosclerosis

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Using surface and deep seawater collected in the sea area of Muroto Cape (Kochi, Japan), desalinated drinking samples of about 1200 hardness were prepared and examined for the effects on the prevention of atherosclerosis in dietary induced hyperlipidemia rabbits. The plasma LDL cholesterol level was lower in the deep seawater group than in the surface seawater group. GPx activity was significantly higher in the deep seawater group than in the control group, while there was no difference between the surface seawater and control groups. The level of LPO was also significantly lower in the deep seawater group than in the control group. The Sudan IV lipid stained area ratio on the inner surface of the aorta was significantly lower in the deep seawater groups than in the control group, while there was no difference between the surface seawater and control groups. The oil red O stained cross section of the aorta in the control and surface seawater administration group foam cells had accumulated to form thick layers, while in the deep seawater administration group, the degree of their accumulation was very low. These results suggested that the deep seawater was useful for the prevention of hyperlipidemia and arteriosclerosis compared to the surface seawater, and it was found that reduction of the LDL cholesterol level and enhancement of GPx activity were involved in its effects.

Key words deep seawater; surface seawater; atherosclerosis; aorta; glutathione peroxidase

Seawater has been used for thalassotherapy and atopic dermatitis for a long time in medical treatment. However, there are still many obscure points in the scientific evidence about the effective of medical treatment.

Deep seawater at depths between about 500 m and 1000 m circulates around the world.[1)] Deep seawater is known to have such characteristics as being clean and rich in mineral components compared to surface seawater,[2—4)] and application has been attempted in many fields.[5—11)] In health food and medical fields, deep seawater has been processed into drinking water, and various beneficial effects have been suggested, but they have not been confirmed scientifically. In particular, differences between surface and deep seawater remain unqualified.

We reported previously that deep seawater was effective for hyperlipidemia and atherosclerosis prevention.[12)]

In this study, we prepared drinking water samples containing the same amounts of major mineral constituents Na, K, Ca, and Mg, with hardness of 1200, by filtering with a semipermeable membrane using surface and deep seawater collected in the sea area of Muroto, Kochi Prefecture, known for the up-flow of deep seawater, and examined the effects of the drinking water samples on the prevention of a typical lifestyle-related disorder, hyperlipidemia and atherosclerosis.

MATERIALS AND METHODS

Animals Male Japanese white rabbits weighing 1.8 to 2.0 kg (Shimizu, Kyoto, Japan) were used in this study. These animals were acclimatized on a 12 h light/dark cycle in a humidity- and temperature-controlled facility and allowed free access to food and water for 1 week before the experiment.

Deep and Surface Seawater Deep seawater pumped up from a depth of 374 m off Muroto Cape (Kochi, Japan) was separated into desalinated water and concentrated by a semipermeable membrane. Various concentrations of water (with sodium eliminated *etc.*) were added to the desalinated water, and deep seawaters were prepared. (hardness of about 1200; Muroto Marinefood Corporation, Odanikokufun Corporation) The surface seawater of the Muroto Cape ocean space was taken and same process was performed to prepare the drinking water (hardness of about 1200; Kochi Prefectural Deep seawater Laboratory).

The mineral-ingredient content of each drinking seawater is shown in Table 1.

Administration of Deep or Surface Seawater Cholesterol fed rabbits were divided into three groups and medicated with 150 ml/d of deep seawater, surface seawater and distilled water in a water-supply bottle *ad libitum*, and fed

Table 1. Mineral Ingredient Content of Seawater

Mineral ingredient	Deep seawater	Surface seawater
Na (mg/l)	51.3	50.2
K (mg/l)	20.0	19.9
Ca (mg/l)	83.5	99.8
Mg (mg/l)	241	237
Cl (mg/l)	473	589
SO_4^{2-} (mg/l)	454	393
Hardness[a)]	1199	1225

a) Hardness: Ca (mg/l)×2.5+Mg (mg/l)×4.1.

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0.25% cholesterol containing diet (CE-2, Clea, Osaka, Japan) for 12 weeks.[12—14)]

The distilled water administration group was used as the control. Body weight was measured weekly.

Measurement of Biochemical Values Blood samples were collected from the ear vein of each rabbit after 12 weeks and centrifuged to obtain sera. Plasma AST, ALT activities and levels of Total cholesterol and HDL cholesterol were measured using Fuji Drichem (Fujifilm Medical Co. Ltd., Japan).[12)] The LDL cholesterol value was measured using MIRACLE ACE 919 (Nipro Co. Ltd., Japan).[15—17)]

Measurement of Plasma Antioxidant Enzyme Activity Measurement of plasma superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activities were carried out using the SOD Assay Kit-WST (Dojindo Molecular Technologies Co. Ltd., Japan)[18,19)] and glutathione peroxidase assay kit (Cayman Co. Ltd., U.S.A.), respectively.

Measurement of Lipid Peroxide Measurement of Plasma lipid peroxide was carried out using lipid peroxide test Wako (Wako Co. Ltd., Japan).[20)]

Morphometric Analysis for Atherosclerotic Lesions At the end of the study period, ascending thoracic aortas were removed after 10% formalin perfusion through the inferior vena cava under anesthesia (pentobarbital, 1 mg/kg) and opened longitudinally. Then they were fixed with 10% formalin for histopathological examination. The aortas were next stained with Sudan IV to detect lipid deposition on the intima. Surface involvement was measured according to the following formula.[21)] Surface involvement (%)=Sudan IV positive lesions area/Aortas total surface area $\times 100$.

Pathologic Histological Observation Aortic samples for histology were obtained from 3 sites at the first bifurcation of the intercostals artery, just above the bifurcation of the celiac artery, and the mostly thickened lesions in the thoracic aorta. They were embedded in paraffin, and sectioned 5 μm-thick. Atherosclerotic lesions were examined histologically after staining with oil red stain.[21)]

Statistical Analysis Significant differences between groups and within groups were compared with a one-way ANOVA. When a significant difference was accepted between groups, it was authorized using an unpaired *t*-test (two tail).

RESULT

Effect of Seawater Administration on Body Weights in the Cholesterol Fed Rabbits All the rabbits survived the experimental period. There were no remarkable differences in the body weights at the control, deep seawater and surface seawater administration groups (data not shown).

Effect of Seawater Administration: Biochemical Values in the Cholesterol Fed Rabbits Differences were not observed between the administration groups for biochemical examination values of liver function such as AST and ALT (data not shown).

Biochemical examination values of plasma lipid after administration of seawater for 12 weeks are shown in Fig. 1.

Differences were observed between the administration groups in the total and LDL cholesterol levels. Total and LDL cholesterol levels of cholesterol fed rabbits in all administration groups increased.

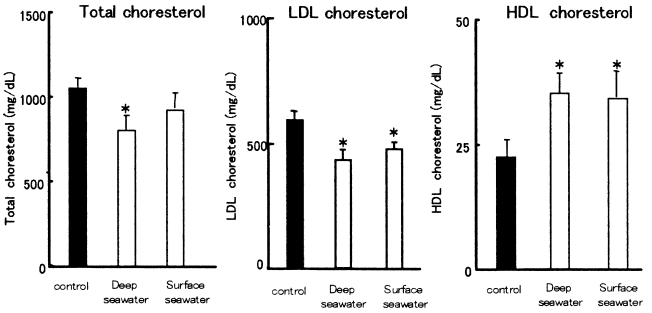

Fig. 1. Effect of Seawater on Plasma Cholesterol Concentration in the Rabbits Fed Cholesterol for 12 Weeks

Each column represents the mean±S.E. of six—seven rabbits. $*p<0.05$, significantly different from the results of control (distilled water).

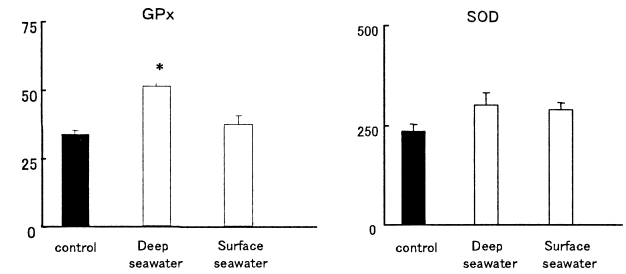

Fig. 2. Effect of Seawater on Plasma Antioxidant Activity in the Rabbits Fed Cholesterol for 12 Weeks

Each column represents the mean±S.E. of seven rabbits. $*p<0.01$, significantly different from the results of control (distilled water).

The plasma total cholesterol level in the cholesterol fed rabbits was significantly lower in the deep seawater administration group (893.0±95.0 mg/dl) than in the control group (1084.0±107.0 mg/dl) and the level was lower in the deep seawater administration group than in the surface seawater administration group (1027.6±93.3 mg/dl).

The plasma LDL cholesterol level in the cholesterol-fed rabbits was significantly lower in the deep (433.0±40.3 mg/dl) and surface seawater administration groups (477.2±25.4 mg/dl) than in the control group (586.6±36.2 mg/dl), and the level was lower in the deep seawater administration group than in the surface seawater administration group.

On the other hand, the plasma HDL cholesterol level in the cholesterol fed rabbits was significantly higher in the deep (35.3±5.1 mg/dl) and surface seawater administration groups (45.9±13.0 mg/dl) than in the control group (22.7±3.5 mg/dl).

Effect of Seawater Administration on Plasma Antioxidant Enzyme Activity in the Cholesterol Fed Rabbits Plasma antioxidant activity values in the cholesterol fed rabbit after administration of sea water for 12 weeks are shown in Fig. 2.

In the plasma SOD activity, differences were not observed between the administration groups (deep seawater group: 300.0±31.5 nmol/mg protein, surface seawater group: 289.7±17.6 nmol/mg protein, control group: 235.3±18.7 nmol/mg protein).

The plasma GPx activity value in the cholesterol-fed rabbits was significantly higher in the deep seawater administration group (51.1±1.9 nmol/min/mg protein) than in the control group (33.8±1.5 nmol/min/mg protein). Otherwise,

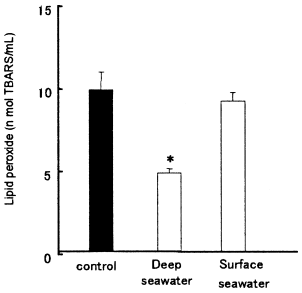

Fig. 3. Effect of Seawater on Plasma Concentration of Lipid Peroxide in the Rabbits Fed Cholesterol for 12 Weeks

Each column represents the mean±S.E. of seven rabbits. $*p<0.01$, significantly different from the results of control (distilled water).

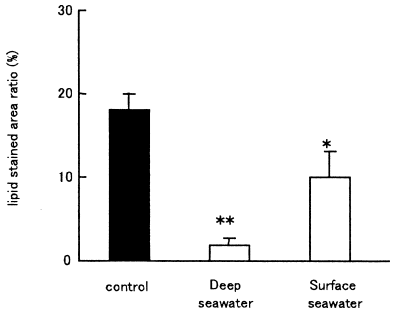

Fig. 4. Effect of Seawater on the Sudan IV Lipid Stained Area Ratio on the Inner Surface of the Aorta in the Rabbits fed Cholesterol for 12 Weeks

Each column represents the mean±S.E. of seven rabbits. $**p<0.01$, $*p<0.05$, significantly different from the results of control (distilled water).

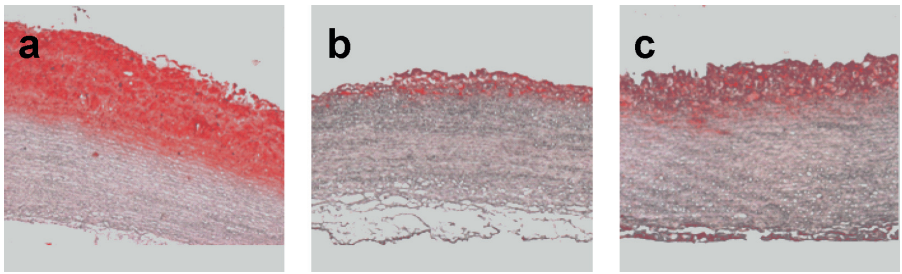

Fig. 5. Oil Red O-Stained Cross-Section of the Aorta

a, control; b, deep seawater; c, surface seawater.

differences were not observed between the control group and the surface seawater group (37.2±3.2 nmol/min/mg protein).

Effect of Seawater Administration on Plasma Lipid Peroxide in the Cholesterol Fed Rabbits Plasma lipid peroxide values in the cholesterol fed rabbits after administration of sea water for 12 weeks are shown in Fig. 3.

The plasma lipid peroxide value in the cholesterol fed rabbits was significantly lower in the deep seawater administration group (4.7±0.3 nmol TBARS/ml) than in the control group (9.9±1.0 nmol TBARS/ml) and the level was lower in the deep seawater administration group than in the surface seawater administration group (9.1±0.5 nmol TBARS/ml).

Pathologic Histological Observations in the Aortas The Sudan IV lipid stained area ratios on the inner surface of the aorta are shown in Fig. 4.

The Sudan IV lipid stained area ratio was significantly lower in the deep seawater administration group (1.8±0.9%) and surface seawater administration groups (10.1±3.1%) than in the control group (18.0±2.0%) and the level was lower in the deep seawater administration group than in the surface seawater administration group.

The oil red O stained cross sections of the aorta are shown in Fig. 5.

In the oil red O stained cross section of the aorta, the control and surface seawater administration groups, macrophages that had phagocytosed Oxidized LDL and became foam cells had accumulated to form thick layers, while in the deep seawater administration group, the degree of their accumulation was very low.

DISCUSSION

The experimental animals were rabbits which had been fed cholesterol for 12 weeks, and the drinking water samples prepared from deep and surface seawater and cholesterol were simultaneously fed. It was found that the deep seawater was effective in suppressing increases in LDL cholesterol, increasing antioxidases in the plasma, suppressing lipid deposition on the inner wall of the aorta, and suppressing foam cell formation compared to the surface seawater.

The deep seawater had effects on several factors involved in the occurrence of hyperlipidemia and arteriosclerosis. The blood LDL cholesterol level in the cholesterol fed rabbits was significantly lower in the deep and surface seawater administration groups than in the control group to which distilled water was fed, and the level was lower in the deep seawater administration group than in the surface seawater administration group. GPx activity which reflects antioxidant activity was significantly higher in the deep seawater administration group than in the control group, while there was no difference between the surface seawater administration and control groups. These results suggested that the deep seawater suppressed increases in blood LDL cholesterol, enhanced GPx activity, and suppressed the generation of oxidized LDL,[22—30] which markedly influences the occurrence of arteriosclerosis. The level of lipid peroxide, most of which are considered to be oxidized LDL, was also significantly lower in the deep seawater administration group than in the control group The Sudan IV lipid stained area ratio on the inner surface of the aorta was significantly lower in the deep and surface seawater administration groups than in the control group, and it was lower in the deep seawater administration group than in the surface seawater administration group. In the oil red O stained cross section of the aorta in the control and surface seawater administration groups, macrophages that had phagocytosed Oxidized LDL and

become foam cells had accumulated to form thick layers, while in the deep seawater administration group, the degree of their accumulation was very low.

Moreover, in the surface seawater administration group, Although plasma antioxidant activity not increased compared with the control group, the Sudan IV lipid stained area ratio decreased. This fact was guessed from this reason having had the plasma GPx and SOD activity of the surface seawater administration group in the upward tendency that the antioxidant activity in the main artery has increased than the control group. The antioxidant activity in the main artery has not been measured since the dyeing experiment was conducted this time, so we think that a detailed examination will be required in the future.

In conclusion, the deep seawater was useful for the prevention of hyperlipidemia and arteriosclerosis compared to the surface seawater, and it was found that reduction of the LDL group cholesterol level and enhancement of GPx activity were involved in its effects. The difference between deep and surface seawater in the content of trace minerals which participate in antioxidant activity was considered to cause the difference in the effects.[31)]

We consider carrying out detailed examination about the various trace element contents of deep and surface seawater. Furthermore, we conduct the experiment which administrated deep or surface seawater for the animal model which lacked various trace elements (selenium, zinc, *etc.*), and consider both relationship.

This study indicated the usefulness of deep seawater for the prevention of lifestyle-related disorders, and differences in the effective of deep and surface seawater.

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