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Effects of red algae cultivated with deep-sea water on the oxidation–reduction status of liver, lung, brain, and bone metabolism in SAMP1 and SAMR1

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Abstract. We investigated the potential of red algae cultivated with deep-sea water (D) as dietary anti-oxidants to ameliorate the redox levels in the main organs and in bone metabolism using SAMP1 and its control SAMR1, in comparison with the same algae cultivated with surface seawater. Only the D group showed marked increases of glutathione (GSH) levels in the liver (R1, 134%) and brain (R1, 128% and P1, 126%) as well as activities of GSH non-dependent peroxidase in the liver (R1, 138%) and the brain (R1, 117% and P1, 112%) but not GSH-dependent peroxidase. The D diet also exhibited beneficial effects on bone metabolism; elevations of femoral calcium, phosphorus, hydroxyproline for collagen, hexosamine for polysaccharides, and suppression of urinary excretion of hydroxyproline as an index of increased bone resorption. A three-point bending test showed that the D diet increased the stiffness and the strength of the femur, which correlated with increases in femoral calcium and phosphorus contents. The results suggest that red algae of the *Gracilaria* sp. cultivated with deep-sea water has the potential to ameliorate degenerative diseases of aging.

Keywords: Deep-sea water; Red algae; Bone metabolism; Anti-oxidants; SAMP1

1. Introduction

The free radical theory of aging has received widespread attention, in which the deleterious effects of reactive oxygen species (ROS) produced through aerobic metabolism are responsible for functional deterioration associated with aging. We investigated the potential of red algae, *Gracilaria tikvahiae*, as a dietary anti-oxidant to ameliorate the aging process itself indicated by the redox levels of the main organs and bone metabolism

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using SAMP1 and its control SAMR1. This algae has a fast and stable growth rate and is expected to be an efficient resource of functional food containing various nutrients including a fluorescent red pigment and β-carotene which has about 100-fold faster reaction constant in scavenging singlet-oxygen than α-tocopherol [1] in addition to various minerals accumulated from deep-sea water when the algae is cultivated with it.

2. Material and methods

One-month-old male mice, SAMP1 and SAMR1, were maintained on the basal diet (C) or on the same diet containing 5% lyophilized powder of red algae cultivated with either deep-sea water (D) or surface-sea water (S), until 7 month old. The latter algae had a straw color in contrast to the former with a wine-red color. All animals had free access to food, which were prepared by pressing the powdered diet into a semi-solid lump. One week before the end of experiment, urine was collected over a 24-h period using our handmade urine traps and the urinary contents of hydroxyproline (OHpro) [2], calcium (Ca), and phosphorus (P), and creatinine were measured. Organs were obtained from mice after 16 h of fasting. The glutathione (GSH) [3] and GSH non-dependent peroxidase (POD) [3] were measured using fresh tissues, and lipid peroxide (TBARS) [3] and GSH-dependent peroxidase (GSH-pex) [3] were assayed using frozen tissues.

3. Results and discussion

There were no changes in the lipid peroxide contents in liver, lung, and brain among the three groups in two strains. As shown in Fig. 1, only the D group showed marked

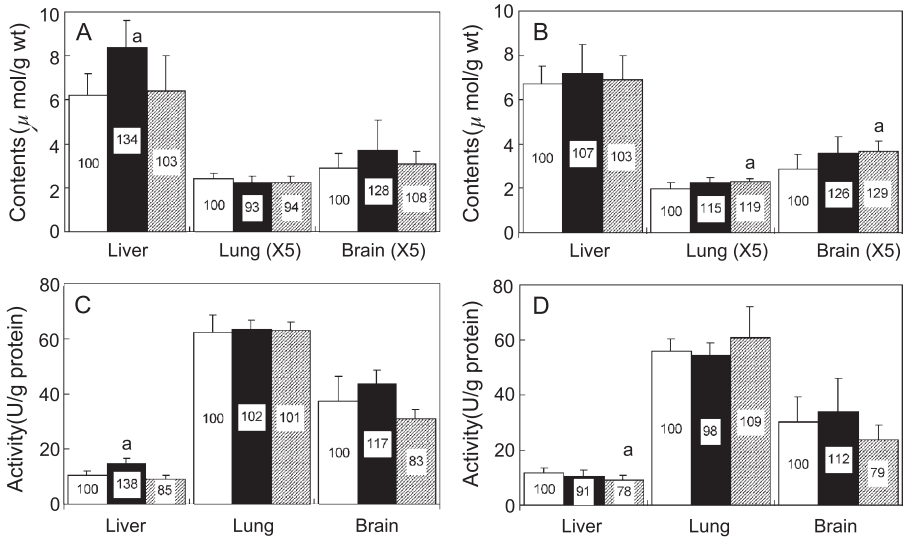

Fig. 1. Glutathione contents (A, B) and peroxidase activity (C, D). (A and C) SAMR1, (B and D) SAMP1. Range bars of the C group (open bars), the D group (shaded bars), and the S group (hatched bars) represent means ± S.D. Significant difference at a: $p < 0.05$ against the C group.

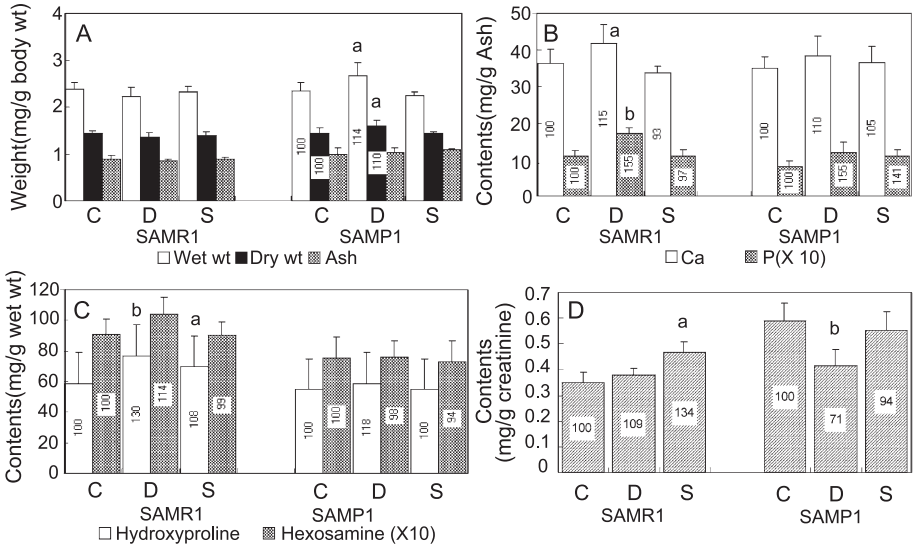

Fig. 2. Femoral weights (A), Ca and P contents (B), hydroxyproline and hexosamine contents (C), and hydroxyproline in 24-h urine (D). Significant difference at a: $p < 0.05$, b: $p < 0.01$ against the C group.

increases in the GSH levels in the liver (R1, 134%) and brain (R1, 128% and P1, 126%) as well as the activities of POD in the liver (R1, 138%) and the brain (R1, 117% and P1, 112%) but not GSH-pex. POD is known to be a major anti-oxidative enzyme in the brain [4]. GSH is also a major endogenous anti-oxidant that declines with age in humans [5] and mice [6]. This decline is linked to cardiovascular diseases and neurodegenerative diseases

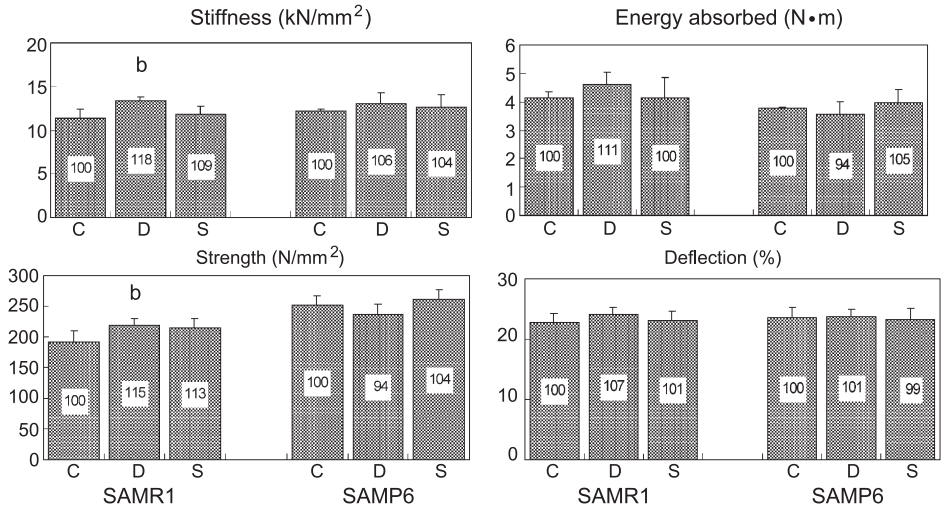

Fig. 3. Mechanical parameters of the femur from SAMP6 and SAMR1. Significant difference at b, $p < 0.01$ against the basal diet control group C.

such as Parkinson's [7] and Alzheimer's disease since sulfhydryls are major component of the cellular antioxidant defenses to ROS and secondary metabolites. The D diet also exhibited beneficial effects on bone metabolism; elevations of femoral Ca (R1, 115% and P1, 110%), phosphorus (R1 and P1, 155%), collagen analyzed by hydroxyproline (R1, 130% and P1, 108%), polysaccharides analyzed by hexosamine (R1, 114% and P1, 98%), and suppression of urinary excretion of hydroxyproline (P1, 71%) which is regarded as an index of increased bone resorption (Fig. 2). A three-point bending test indicated that the D diet increased the stiffness and the strength of the femur (Fig. 3), correlating with the increases in Ca and P contents (Fig. 2) reported previously [8]. The present results suggest that the red algae *Gracilaria* sp. has potential as a dietary antioxidant to ameliorate degenerative diseases of aging [9] such as neurodegenerative diseases and osteoporosis.

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