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Seaweed Prevents Breast Cancer?

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To investigate the chemopreventive effects of seaweed on breast cancer, we have been studying the relationship between iodine and breast cancer. We found earlier that the seaweed, *wakame*, showed a suppressive effect on the proliferation of DMBA (dimethylbenz(*a*)anthracene)-induced rat mammary tumors, possibly via apoptosis induction. In the present study, powdered *mekabu* was placed in distilled water, and left to stand for 24 h at 4°C. The filtered supernatant was used as *mekabu* solution. It showed an extremely strong suppressive effect on rat mammary carcinogenesis when used in daily drinking water, without toxicity. *In vitro*, *mekabu* solution strongly induced apoptosis in 3 kinds of human breast cancer cells. These effects were stronger than those of a chemotherapeutic agent widely used to treat human breast cancer. Furthermore, no apoptosis induction was observed in normal human mammary cells. In Japan, *mekabu* is widely consumed as a safe, inexpensive food. Our results suggest that *mekabu* has potential for chemoprevention of human breast cancer.

Key words: Breast cancer — Chemoprevention — *Mekabu* (seaweed) — Apoptosis

Chemoprevention of breast cancer has received much attention in Europe and the U.S. because of the high incidence of this cancer (1 in 4–8 persons) and the strong hereditary component.[1–4] Tamoxifen is accepted as a chemopreventive agent following the results of the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 study.[5] However, despite the increasing incidence of breast cancer in Japan, the use of chemopreventive agents is so far forbidden. We have been studying the relationship between iodine and breast cancer for 10 years, trying to determine whether iodine is useful for chemoprevention of breast cancer.[6–8] There have been several reports on the relationship of iodine and breast cancer,[9–11] but the present paper is the first concerning breast cancer chemoprevention by seaweed.

We first examined diluted Lugol's solution, an inorganic iodine solution that has been employed as a clinical test reagent for humans. Inorganic iodine suppressed the proliferation of DMBA (dimethylbenz(*a*)anthracene)-induced rat mammary tumors, and orally administered iodine eventually reached the tumor tissues.[7] On the basis of these results, we examined the effect of seaweed, a natural food containing iodine.[8] In the same report, we presented the results of our experiments using the seaweed *wakame*, which was given to rats mixed into usual rat feed at 1 and 5% by weight. *Wakame* showed a strong suppressive effect on the proliferation of DMBA-induced rat mammary tumors even in the 1% *wakame* group, and apoptosis induction was thought to be a possible mechanism of this effect. However, this dose, if scaled up to the human body weight, is too large to be practicable.[8]

In the present experiments, *mekabu* was used instead of *wakame*. It contained almost the same amount of iodine as the *wakame* used in our former experiments (5.9 vs. 5.7 mg/100 g), but is cheaper and easier to process than *wakame*. In the *in vivo* experiments, a single dose of 20 mg/body of DMBA (Wako Junyaku Kogyo, Tokyo), a rat mammary carcinogen, was dissolved in sesame oil and given by gastric intubation to two randomly divided groups of eight-week-old female SD (Sprague-Dawley) rats (Japan SLC, Inc., Shizuoka) weighing 180 to 200 g (n=12 for each group). All rats were fed with CE-2 (CLEA Japan, Inc., Tokyo). Beginning 1 week after DMBA administration, the 2 groups were given tap water and *mekabu* solution. *Mekabu* solution was prepared as follows using powdered *mekabu* (Riken Vitamin, Inc., Tokyo), which contains 5.9 mg/100 g of iodine, 889 mg/100 g of calcium, 3.16 g/100 g of potassium and 1.65 g/100 g of NaCl. Powdered *mekabu* 1.5 g was mixed with 1000 ml of distilled water and stirred. After 24 h at 4°C,

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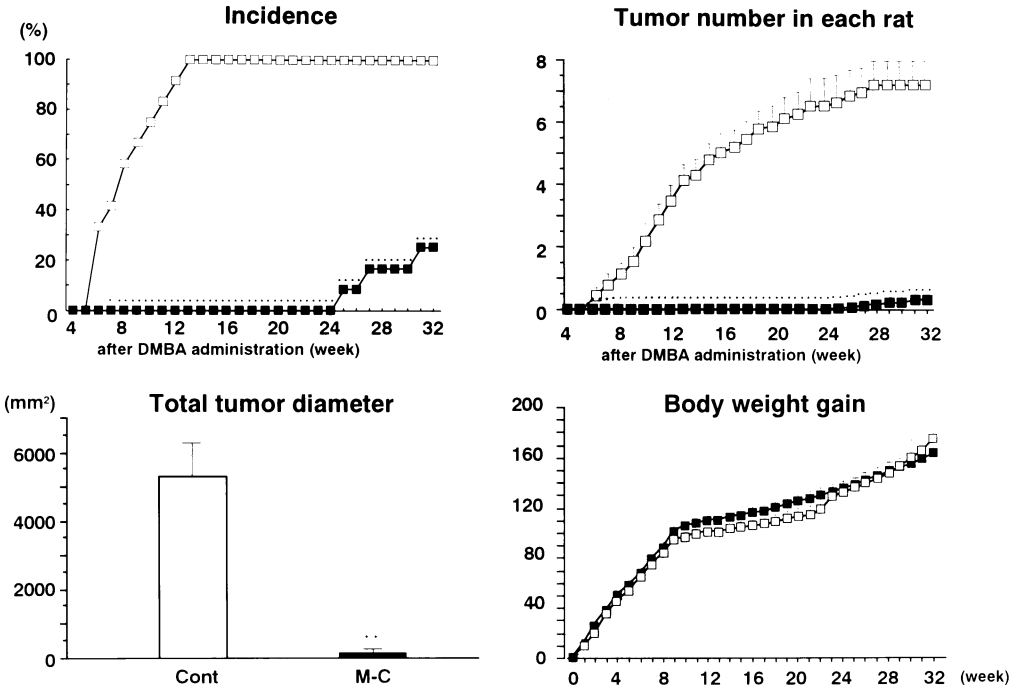

Fig. 1. Significant differences were noticed in the incidence of mammary tumors between the control and *mekabu* group each week. There were significant differences between the 2 groups in the number of tumors per rat, as well as a significant difference between the 2 groups in total tumor diameter at 32 weeks. Throughout the 32 experimental weeks, all rats in both groups showed the same weight gain in a subchronic toxicity test. ** $P<0.01$ vs. control group. □ control, ■ *mekabu* group.

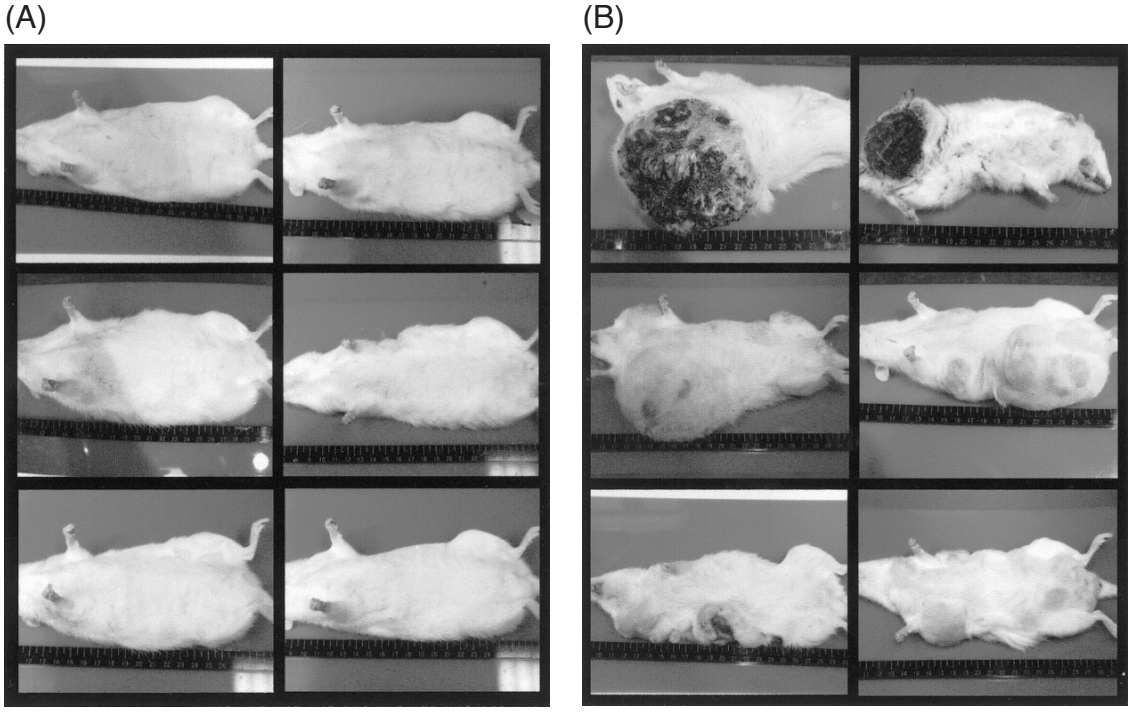

Fig. 2. Mammary tumors were not observed macroscopically in rats of the *mekabu* group (A) despite huge tumors in the control group (B).

the mixture was filtered through 3 kinds of nylon mesh (7.0, 4.5 and 0.45 μm), and the filtrate was used as *mekabu* solution. Weekly changes in rat body weight, incidence and the number of mammary tumors in each rat were then observed for 32 weeks. The entire mammary tumors of each rat were removed, and the total tumor diameter was measured at the end of the 32-week experiment. The incidence of mammary tumors in rats of the group given *mekabu* was extremely low (Figs. 1, 2). Throughout the experiment, the number of tumors in the *mekabu* group was significantly lower than in the control group. (Figs. 1, 2). The total tumor diameter in the *mekabu* group was significantly smaller than in the control group. Histologically, the mammary tumors were cystic adenocarcinoma, and tumors in the *mekabu* group showed a decreased density of epithelial cells and fibrosis. In a separate subchronic toxicity test experiment, 24 normal 8-week-old female SD rats were randomly assigned to two groups given the same 2 kinds of drinking water without DMBA administration. They were then monitored for 32 weeks by measuring weekly body weight changes. No significant difference in weekly body weight changes was seen between the 2 groups (Fig. 1).

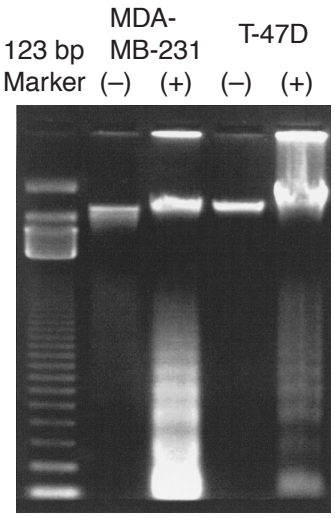

Fig. 3. DNA ladder formation was seen in 2 human breast cancer cell lines after 96-h culture with (+) *mekabu* solution. (–), without *mekabu*.

In the *in vitro* experiments, 0.5, 1.0 or 2.0 g of powdered *mekabu* was dissolved in 150 ml of distilled water, and *mekabu* solution was prepared by the same filtration procedure mentioned above. One ml of *mekabu* solution

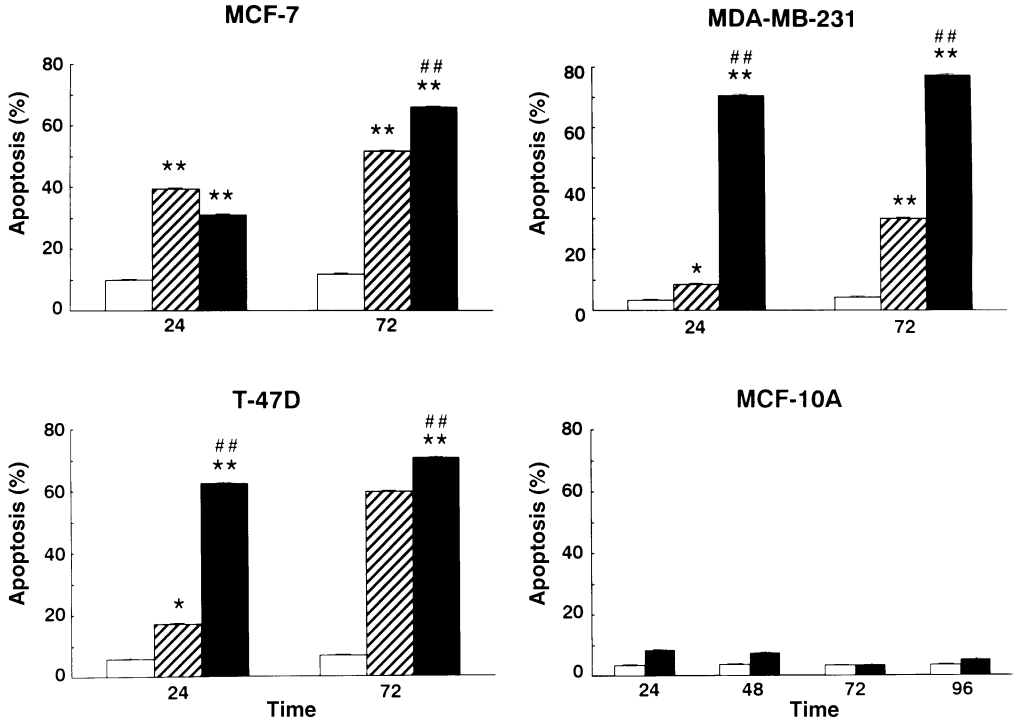

Fig. 4. *Mekabu* (■) showed significantly higher apoptosis induction compared with both the control (□) and 5FU (▨) after 72-h culture in 3 kinds of human breast cancer cell line. At 24-h culture, *mekabu* showed significantly higher apoptosis induction than the control and 5FU except in MCF-7 cells. Human normal mammary cells (MCF-10A) showed no differences in apoptosis induction between the control and *mekabu*. ** $P<0.01$ vs. control, ## $P<0.01$ vs. 5FU.

was added to culture medium containing 5×10^6 T-47D cells. The 1.0 g *mekabu* group showed higher apoptosis induction ability than the 0.5 g *mekabu* group and almost the same ability as the 2.0 g *mekabu* group. Therefore, 1.0 g *mekabu* solution was used in the following experiments. MCF-7, T-47D, and MDA-MB-231 human breast cancer cell lines and normal human mammary cells (MCF-10A),[12, 13] were used. Apoptosis induction ability was measured by conventional flow cytometry.[14] DNA fragmentation was analyzed by conventional gel electrophoresis with agarose gel using an Apoptosis Ladder Detection Kit (Wako Pure Chemical Ind., Ltd., Osaka).[15] *Mekabu* showed strong apoptosis induction in all 3 breast cancer cell lines, and clear DNA fragmentation was seen in T-47D and MDA-MB-231 cell lines (Fig . 3). Next, we compared apoptosis induction by *mekabu* and by 15 μM 5FU (5-fluorouracil), a chemotherapeutic agent frequently used in human breast cancer clinics. Raymond *et al*. reported that the IC_{50} value of 5FU in MCF-7 breast cancer cell line was 23 ± 3.2 μM.[16] *Mekabu* showed stronger apoptosis induction than that of 5FU in all 3 human breast cancer cell lines (MCF-7, T-47D and MDA-MB-231). However, normal human mammary cells showed no apoptosis induction (Fig. 4).

The significance of differences was assessed using JMP statistical software for Macintosh (SAS Institute Inc., Cary, NC). ANOVA and Tukey-Kramer HAD (honestly significant difference) were used to compare all pairs. A P value of less than 0.05 was taken as indicative of a significant difference.

In our experiments *in vivo*, rats drank 27 ml of water a day, and their average body weight was 250 g. The corresponding dose for humans, based on body weight, would be about 7 g of *mekabu* a day. It might be reasonable to consume this amount in the form of *mekabu* solution. Regarding the mechanism of apoptosis induction by *mekabu*, iodine may play a role in enhancing the function of superoxide dismutase. However, *mekabu* contains many other components besides iodine. There are several reports investigating the chemopreventive effects of catechin, vitamins, etc.[17, 18] We are now conducting an analysis of the water-soluble components of *mekabu* that are effective for chemoprevention and apoptosis induction.

In conclusion, *mekabu* may well be applicable for chemoprevention of human breast cancer.

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