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Ulva lactuca : A Potential Seaweed for Tumor Treatment and Immune Stimulation

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Abstract This is the first report on the antitumor and immunostimulating activities of *Ulva lactuca*. Using the WSM (water-soluble fraction of a methanol extract from *Ulva lactuca*), a concentration of 140 g/mL was found to inhibit 50% of the human leukemia cell line U937 in growth, while splenocyte growth was stimulated up to a concentration of 100 μg/mL. In addition, NO production by a macrophage cell line (RAW 264.7) and alkaline phosphatase activity in mouse splenocytes were both stimulated with 10 μg/mL of WSM. Dose-dependent patterns were observed on all three cell-lines. Accordingly, the current results indicate that *Ulva lactuca* may be useful as a natural antitumor and immunostimulating agent.

Keywords: *Ulva lactuca*, methanol extract, U937, nitric oxide, macrophage, splenocyte

Antitumor chemotherapeutic drugs produce neutropenia as a side effect. In addition, the administration of such drugs injures blood-forming functions, including cells that are crucial in maintaining the body's defense systems, thereby potentially accelerating the risk of tumor metastasis and fungal infection [1,2].

Macrophages are important cells that play key roles in the immune system defense, including the phagocytosis of pathogens and production of many cytokines. Plus, nitric oxide (NO) has also been recognized as a mediator with similar functions to cytokines [3,4].

Research has already been conducted to identify a natural biologically active compound that possesses antitumor, antimicrobial, enzyme inhibiting, lymphocyte stimulating, and radical scavenging properties with low side effect [5,6]. As a result, molecules from marine organisms have been found to exhibit a high biological activity, especially seaweeds, which are a traditional food, known to cure various diseases and maintain health, and recently found to produce immunomodulating and antitumor activities [7-9]. However, most previous studies have used animal models, therefore, the current study is the first investigation of the effects of *Ulva lactuca* on a human leukemia tumor cell line, murine lymphocytes, and splenocytes *in vitro*.

The biomass of *Ulva lactuca* was harvested from the Busan coastline in Korea and concentrated 37.1-fold by boiling in methanol, filtration, evaporation, and lyophilization. Sterilized distilled water was then added before a cell growth assay, NO production enumeration, and splenocytes stimulation. The human leukemia cell line (U937), murine macrophage cell line (RAW 264.7 cells), and splenocytes were all grown in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) containing 2 mM glutamine, 10% heat-inactivated fetal bovine serum (Bio Whittaker, Walkersville, MD, USA), penicillin (100 units/mL), and streptomycin (100 μg /mL) at 37°C in 5% CO_2 [10]. All chemicals were purchased from Sigma Chemical Co.

An MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed using 96-well plates (Roche, Indianapolis, IN, USA) following the previously described procedure of Hyun *et al.* [11]. The amount of NO in the cultured medium of macrophages was determined based on measuring the nitrite, as the stable end product of NO [12].

The cell lysates were measured for alkaline phosphatase (ALP) activity to estimate the effect of the water-soluble fraction of a methanol extract from *Ulva lactuca* (WSM) on the splenocyte activity, while colorimetric assays [13] were used to assess the WSM activity. The stimulation index (SI) for the assay was defined as the ratio of the absorbance signal in the control to that in the WSM-stimulated cells and calculated as follows:

$$SI = (S\text{-}C)/C$$

where S and C represent the absorbance values for the WSM-stimulated and control cells, respectively.

More than 25 μg/mL of WSM (water soluble fraction from *Ulva lactuca* methanol extract) was found to inhibit cell proliferation of human leukemia U937 cells *in vitro* (Fig. 1A). Growth of U937 cells was decreased in pro-

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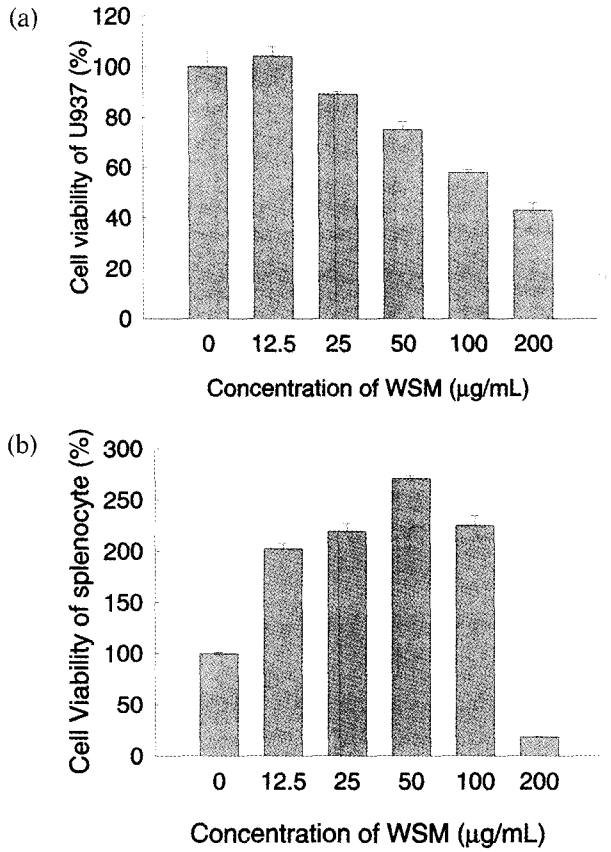

Fig. 1 Viability of human leukemia cell line U937 (A) and splenocytes of ICR mouse, (B) to the variable concentration of methanol extract of *Ulva lactuca*.

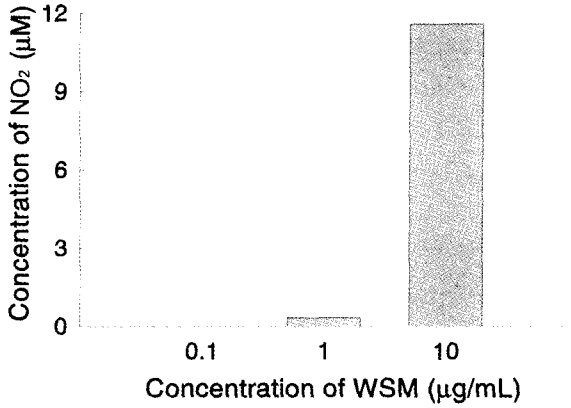

Fig. 2. NO concentration of Raw 264.7 macrophage to the variable concentration of methanol extract of *Ulva lactuca*.

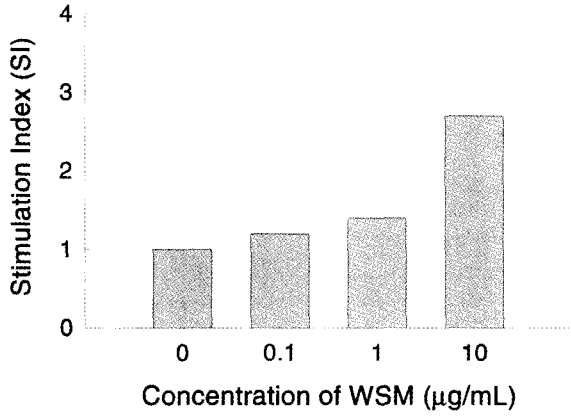

Fig. 3. Alkaline phosphatase activities of splenocytes to the variable concentration of methanol extract of *Ulva lactuca*.

portion to the concentration of WSM. About 10% of the cells were inactivated with 25 μg/mL of WSM, while half of the leukemia cells were inactivated with 140 μg/mL of WSM. Although the growth of the U937 cells seemed to increase with 12.5 μg/mL of WSM, the standard deviation was within a range of 0 μg/mL of WSM.

Meanwhile, the splenocyte growth increased more than 2-fold with an increase in the WSM concentration from 25 and 100 μg/mL, however, at 200 μg/mL the splenocyte growth became inhibited (Fig. 1B). Therefore, these results show that 25 to 100 μg/mL of WSM inhibited the growth of tumor cells while stimulating the growth of splenocytes *in vitro*.

Since a purified material is expected to be more efficient than the crude extract used in the current study [14, 15], further efforts are being made to separate and fractionate the effective material for comparison.

Murine splenocytes and macrophages were chosen for their potential ability to enhance the immune responses. Macrophages generate reactive oxygen species (ROS) through the partial reduction of oxygen, one of which is nitric oxide (NO). In the current study, macrophage activation caused an oxidative burst in the cultured medium (Fig. 2), implying a stimulatory effect in the host defense against microbial and viral infection by the activated macrophages. The macrophages exposed to WSM produced increasing amounts of NO in a concentration-dependant manner, indicating that the extract from *Ulva lactuca* was an effective NO inducer. At a WSM concentration of 10 μg/mL, the nitrate production was drastically stimulated on a level equivalent to that produced by 50 μg/mL of the extract from the leaves of *P. frutescens* var. *crispa*. [16] based on a similar concentration fold to that used in the current study.

Chung *et al.* [17] previously demonstrated that the antiinfectious and antitumor effects of *Oldenlandia diffusa* are due to its ability to enhance the nonspecific immunologic activity of NO. Hence, it is possible that WSM may have a similar indirect antitumor function as *Oldenlandia diffusa*.

The murine splenocytes exhibited correlated activities according to the WSM concentration (Fig. 3), with very little difference until 1 μg/mL of WSM, yet a high SI value at 10 μg/mL of WSM, which matched the macrophage results. The increased ALP activity was likely caused by the increased number of cells, rather than by functional activation of the cells (Fig. 2). However, the

cell growth was 2-fold with 12.5 μg/mL of WSM, whereas the ALP activity was about 2.7 fold with 10 μg/mL of WSM. Accordingly, it would appear that WSM stimulated an immune response.

This is the first report on the effect of *Ulva lactuca* on U937 (human tumor cell line), macrophages, and splenocytes. As such, the water-soluble fraction of a methanol extract from *Ulva lactuca* exhibited an antitumor effect, stimulated the growth activity of splenocytes, and increased the NO production of macrophages. Consequently, the current results indicate that *Ulva lactuca* has the potential to offer antitumor, antimicrobial, antiviral, and immune stimulating activities under certain conditions *in vivo*. Therefore, further pharmacological investigations of WSM are desirable.

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