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Cytoprotective effects of fucoidan, an algae-derived polysaccharide on 5-fluorouracil-treated dendritic cells

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ABSTRACT

Although chemotherapeutic anticancer agents are effective, they also attack normal immune cells due to a lack of selectivity. 5-Fluorouracil (5-FU) is a representative anticancer agent that induces immunosuppression in cancer patients as a side effect. Fucoidan is an algae-derived sulfated polysaccharide that has recently been recognized as a hematopoietic mobilizer and immunomodulator. In this study, we investigated the cytoprotective effect of fucoidan on dendritic cells (DCs) against 5-FU-induced cellular damage. Several kinds of assays including flow cytometric analysis demonstrated the cytoprotective efficacy of fucoidan. In addition, fucoidan increased the expression of immune-related surface markers on and the alloproliferative capacity of DCs exposed to 5-FU. For investigating action mechanism, the expression levels of apoptosis-related molecules were measured. Taken together, the results of this study suggest that fucoidan, a marine-derived polysaccharide, has cytoprotective effects on DCs, the most potent antigen-presenting cell type, against 5-FU-induced cellular damage. These results provide valuable information to use fucoidan as an immunostimulatory agent for the chemotherapy of cancer patients.

1. Introduction

Fucoidan is an edible algae-derived sulfated polysaccharide (Anastyuk et al., 2009; Hidari et al., 2008) that is known for its biological activities, including hematopoietic mobilization (Frenette and Weiss, 2000) and immunomodulation. The effects of fucoidan have been demonstrated in a variety of immune cell types, including B lymphocytes (Oomizu et al., 2006) and macrophages (Yang et al., 2006). Such studies indicate that fucoidan inhibits IgE production by B lymphocytes *via* germline transcription and regulates nitric oxide production by macrophages. We recently showed that fucoidan stimulates the immune function of dendritic cells (DCs), the most potent antigen-presenting cell (APC) type (Kim and Joo, 2008).

It is generally accepted that many chemotherapeutic agents for cancer treatment have severe side effects due to toxicity and a lack of specificity for cancer cells. Such fast-growing cells as immune cells and hematopoietic cells are susceptible to the cytotoxicity of various chemotherapeutic anticancer agents; thus, chemotherapeutic agent-induced immunosuppression and a decreased level of leukocytes are frequently seen in cancer patients. Therefore, the development of new adjuvants to attenuate the immunosuppression caused by chemotherapeutic anticancer agents is an important area of cancer research.

In this study, we selected 5-fluorouracil (5-FU), a representative anticancer agent used for chemotherapy in patients. 5-FU has been used in the treatment of a variety of cancer cells, including colon cancer, gastric cancer, pancreatic cancer, and non-small-cell lung cancer (Choi et al., 2005; Ohtsu, 2008; Tanaka et al., 2000). Although it has been used for over 40 years, 5-FU has severe side effects like other chemotherapeutic agents, which limits the dosages used for chemotherapy. It has been shown that 5-FU induces apoptosis in normal cells as well as cancer cells *in vitro* (Sakaguchi et al., 1994). In this study, we investigated whether fucoidan can protect DCs from 5-FU-induced cellular damage including cell death.

2. Materials and methods

2.1. Animals and reagents

C57BL/6, Balb/c mice were purchased from ORIENT BIO (Republic of Korea) and maintained at our animal facility. Both strain female mice, 7- to 12-week old, were used for all experiments. Animal experiments in this study were performed according to the Institutional guideline of Jeju National University for animal use and care.

2.2. Preparation of fucoidan

Fucoidan originated from *Fucus vesiculosus* was purchased (Sigma, St. Louis, MO) and dissolved using phosphate buffered saline. To quantify the endotoxin level in fucoidan preparation, we used QCL-1000® Chromogenic LAL endpoint assay

Abbreviations: 5-FU, 5-fluorouracil; APC, antigen-presenting cell; DCs, dendritic cells; FITC, fluorescein isothiocyanate; imDCs, immature DCs; PI, propidium iodide.

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(Lonza Walkersville, Inc., Walkersville, MD) as followed the manufacturer's manual (Kim and Joo, 2008). The endotoxin level in 100 μg/ml fucoidan preparation was less than the detection limit of the kit (0.1 EU/ml).

2.3. Preparation of bone marrow cell-derived DCs

The bone marrow cells were obtained from the femur and tibia of C57BL/6 mice by flushing and cultured in the presence of 10 ng/ml granulocyte macrophage-colony stimulating factor (Invitrogen, Carlsbad, CA) as described in our previous report (Kim and Joo, 2008). The characterization of DCs was occasionally performed based on flow cytometric analysis.

2.4. Measurement of the DC viability

The cultured DCs were seeded at a concentration of 5×10^4 cells/200 μl/well in 96-well culture plates for cell viability/proliferation assays, using Cell Counting Kit-8® solution (CCK-8; Dojindo, Gaithersburg, MD) or 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) solution. For CCK-8 assay, 10 μl/well of CCK-8 solution was added into the cultured wells after the indicated treatment and incubated for 4 h. For MTT assay, MTT solution was added into cultured wells at the concentration of 0.5 mg/ml and incubated for 4 h. The crystal violet generated by viable cells was dissolved with 10% sodium dodecyl sulfate (SDS; Sigma) solution for 2 h. The optical density of wells was measured at 450 nm (CCK-8 assay) and 570 nm (MTT assay) using a microplate reader (Molecular devices, Sunnyvale, CA). For checking the viability, DCs were cultured in 6-well culture plates and stained with a trypan blue solution (Invitrogen) to count viable and dead cells.

2.5. Flow cytometric analysis

The staining of cells for flow cytometric analysis was processed as established in our laboratory (Joo, 2003). DCs were treated with biotin-labeled anti-CD11c, anti-H-2^b, anti-I-A^b, anti-CD54, anti-CD86 antibody and then with streptavidin–fluorescein isothiocyanate (FITC) (all from BD Biosciences, San Jose, CA). For measuring the membrane potential of mitochondria, DCs were treated with 10 μg/ml rhodamine 123 (Sigma) for 30 min at room temperature. The stained cells were analyzed by FACSCalibur® and CellQuest® (Beckton Dickinson, Franklin Lakes, NJ).

2.6. Western blot analysis

The expression level of apoptosis-related proteins in DCs was determined by Western blot analysis (Joo et al., 2001). Briefly, DCs were harvested and dissolved in lysis buffer. The protein concentration of cell lysates was determined by using a Bio-Rad protein assay solution. Same quantity of protein was loaded into each lane and electrophoretically separated in 12% SDS polyacrylamide gels. The separated proteins were transferred onto a nitrocellulose membrane and probed using anti-Bcl-2, anti-Bcl-xL, anti-Bax, anti-cIAP-1, anti-cIAP-2 antibody. And then the membrane was treated with horseradish peroxidase-labeled secondary antibody and developed by SuperSignal® West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL) to visualize specific protein bands.

2.7. Enzyme-linked immunosorbent assay (ELISA)

The cultured DCs were treated with 5-FU and fucoidan. The supernatants were collected and used for ELISA. The amounts of TNF-alpha and IL-12 in supernatants were measured by using CytoSet™ kit (Invitrogen) based on the manufacturer's instruction.

2.8. Mixed lymphocyte reaction

Splenocytes were obtained from Balb/c mice (Joo et al., 2001) and used as allogeneic lymphocytes in the co-culture with DCs of C57BL/6 mice. DCs were co-cultured with 2×10^5 cells/well allogeneic splenocytes for 5 days. Before the co-culture, DCs were irradiated by 30 Gy by using the irradiator that was established in Jeju National University. After the co-culture, CCK-8 solution (Dojindo) was used to measure the proliferation capacity of allogeneic splenocytes.

2.9. Statistical analysis

Data were presented as mean ± SD and statistically analyzed by Student *t* test. A *p* value of <0.05 was considered as significant.

3. Results

3.1. The effect of fucoidan on the viability of 5-FU-treated DCs

To study the effect of fucoidan on the viability of DCs treated with 5-FU, we performed viability assays, CCK-8 and MTT assay. Due to the lack of selectivity, 5-FU damaged the survival of DCs. In CCK-8 assay, the optical density of DCs treated with 100 μg/ml 5-FU was lower than that of DCs treated with 30 μg/ml 5-FU. Of note, fucoidan increased the viability of DCs in a concentration-dependent manner up to 20 μg/ml (Fig. 1A). 100 μg/ml fucoidan showed a marginal cytotoxicity on DCs (data not shown), thus 50 μg/ml fucoidan was used for this study. In same experimental setup, MTT assay also showed similar result (data not shown). To confirm the effect of fucoidan on DC viability, a trypan blue staining was performed. The viability of DCs treated with fucoidan and 5-FU was higher than that of DCs treated with 5-FU alone (Fig. 1B). Based on flow cytometric analysis, the percentage of DCs with regular cell size was increased by fucoidan treatment (Fig. 1C). Thus, these results indicate that fucoidan has the cytoprotective effects on DCs against 5-FU in the aspects of viability and cell size.

3.2. The protective effects of fucoidan on 5-FU-induced cellular damage of DCs

For measuring the action potentials of mitochondrial membrane of cells (Bedner et al., 1999), an assay using rhodamine 123 solution was performed. The cells in apoptotic process have lower action potential of mitochondrial membrane than that of

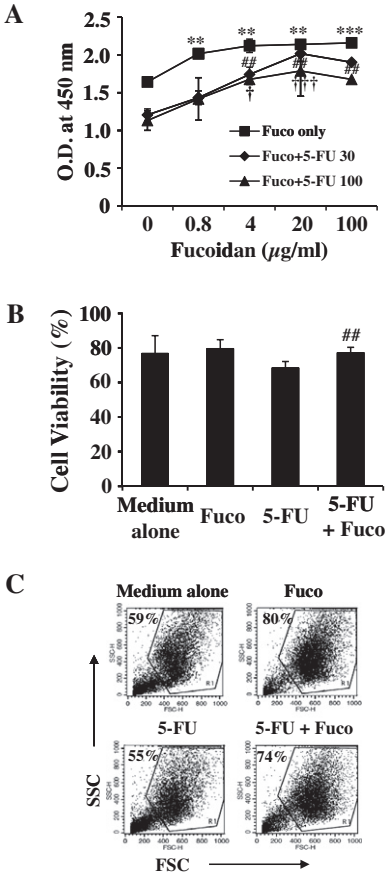

Fig. 1. Effect of fucoidan on DC viability. The cultured DCs were setup and treated as described in Section 2. For an assay using CCK-8 solution (A), DCs were treated with 0–100 μg/ml fucoidan and 30, 100 μg/ml 5-FU. **,***indicate $p < 0.01$, 0.001 compared to control DCs, ##indicates $p < 0.01$ compared to DCs treated with 30 μg/ml 5-FU, and †,†††indicate $p < 0.05$, 0.001 compared to DCs treated with 100 μg/ml 5-FU. For trypan blue exclusion test (B) and cell-size analysis (C), DCs were setup at a concentration of 1×10^6 cells/well of 6-well culture plates and treated with 50 μg/ml fucoidan and 100 μg/ml 5-FU for 2 days. In B, ##indicates $p < 0.01$ compared to DCs treated with 5-FU. Representative data are shown from three independent experiments with similar results.