Simultaneous Treatment of Cancer Cells Lines with the Anticancer Drug Cisplatin and the Antioxidant Fucoxanthin

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Abstract: The anti-oxidative properties and the other health benefits of fucoxanthin (Fx) make it a good candidate for a health food supplement. However, the use of antioxidant supplements in patients undergoing chemotherapy has been widely debated because of concerns that the antioxidants may interfere with the mechanisms of the anticancer drugs. To investigate this concern, we studied the effect of Fx on the anticancer drug cisplatin. Fucoxanthinol (Fxol), which is a deacetylated product produced during intestinal absorption, and other antioxidants (fucoxanthin acetate, astaxanthin, and α-tocopherol) were also included in the experiments. Both Fx and Fxol suppressed the proliferation of cultured cancer cells (Caco-2, Hep G2, and Neuro2a) in a dose-dependent manner, whereas the other antioxidants showed no evident effect on their growth. Moreover, when added to a medium containing cisplatin, none of the studied antioxidants mitigated the inhibitory growth effects of cisplatin. Our results suggested that intake of Fx may help to prevent cancer and that it may not interfere with the mechanisms of anticancer drugs during chemotherapy. However, further studies, using animal models in particular, are required to elucidate these interactions.

Key words: Anticancer agent, carotenoid, fucoxanthinol

INTRODUCTION

Fucoxanthin (Fx) is a major marine carotenoid found in brown seaweeds (Chandini et al., 2008). Recently, an Fx-based functional food has been developed (Mise et al., 2011). Fx exhibits anti-carcinogenic, anti-obesity, anti-inflammatory, anti-angiogenic, and anti-oxidative activities (Okuzumi et al., 1993; Maeda et al., 2005; Shiratori et al., 2005; Sugawara et al., 2006; Heo et al., 2008). Moreover, orally administered Fx is safe in terms of mutagenic potential (Beppu et al., 2009). Though Fx is a useful marine carotenoid, no studies have described the use of the antioxidant Fx in patients undergoing chemotherapy. A great deal of debate has focused on the use of antioxidant supplements in patients undergoing chemotherapy because of the concerns that the antioxidants may interfere with the mechanisms of anticancer drugs and subsequently decrease their efficacy. Some cancer patients have understood from a general perspective that “supplements protect the body from oxidative damage, and the mechanism for protection against chemotherapy is well understood.” However, Gabriella and Andrea (2005) have reported that multiple lines of evidence from extensive human studies are needed before patients are advised to take antioxidants during cytotoxic therapy. Nonetheless, the evidence from different studies has suggested that antioxidant supplements may reduce the toxic side effects of ROS-generating chemotherapies (Block et al., 2008).

Cisplatin is one of the most potent anticancer drugs used for cancer therapy (Boulikas and Vougiouka, 2003) and has been shown to induce nephrotoxicity through oxidative stress (Yao et al., 2007). For investigating the effects of Fx on chemotherapy, the interaction of cisplatin and Fx was investigated. Fucoxanthinol (Fxol), which is a de-acetylated product generated from the hydrolysis of Fx during intestinal absorption, was also investigated for its interaction with cisplatin. Additional antioxidants (fucoxanthin acetate, astaxanthin, and α-tocopherol) were also investigated in order to compare their effects with Fx and Fxol.

Our cultured cell (Caco-2, Hep G2, and Neuro2a) studies showed that Fx and Fxol increased the level of cytotoxicity in a dose-dependent manner. However, fucoxanthin acetate (Fxac), astaxanthin, and α-tocopherol had no evident cytotoxic effects. In addition, although the antioxidants were potent, they did not interfere with the mechanism of action of cisplatin. Further studies will be necessary to clarify the effect of Fx during chemotherapy, especially in animal experiments.

MATERIALS AND METHODS

Fucoxanthin, fucoxanthinol, and fucoxanthin acetate: Fucoxanthin (Fx) was extracted from Undaria pinnatifida, and fucoxanthinol (Fxol) and fucoxanthin acetate (Fxac) were synthesized in laboratory (Fig. 1). Fx was isolated from brown seaweed Undaria pinnatifida in May 2009, as...
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Fig. 1: (a) A fucoxanthin crystal as observed by a light microscope. The scale bar represents 20 μm. (b) An Fx crystal as observed by a stereomicroscope. The scale bar represents 500 μm. (c) The chemical structures of Fxol, Fx, and Fxac previously reported (Kadekaru et al., 2008). Fxol was prepared by hydrolysing Fx with lipase in September 2009, as described previously (Mise et al., 2011). Fxac was prepared from Fx by acetylation in June 2010: Fx (12.8 mg) was dissolved in pyridine (300 μL), mixed with acetic anhydride (180 μL), and incubated at 32°C for 3 h. The reaction mixture was partitioned between diethyl ether and water. The ether layer was evaporated, and the residue was dissolved in methanol (MeOH). The purity of Fx, Fxol, and Fxac was determined by HPLC and monitored at 440 nm. The structures of these compounds were identified by NMR.

Cell culture: The human colonic cancer Caco-2, hepatocarcinoma Hep G2, and rat neuroblastoma Neuro2a cell lines were purchased from the RIKEN BRC Cell Bank. Caco-2 cells were maintained in D-MEM (high glucose) with L-glutamine and phenol red (Wako) containing 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. Hep G2 cells were maintained in Dulbecco's modified Eagle's medium (D-MEM; low glucose) with L-glutamine and phenol red (Wako) containing 10% heat-inactivated foetal bovine serum (FBS, GIBCO), 100 units/mL penicillin, and 100 μg/mL streptomycin (SIGMA). Neuro2a cells were maintained in MEM Earle's liquid (GIBCO) containing 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in a humidified atmosphere with 5% CO₂ at 37°C. This experiment was conducted in January-March 2011.

Cell viability: For all experiments, cells were seeded at a density of approximately 1×10⁴ cells per well containing 100 μL of culture medium in 96-well plates. After 24 h incubation, the medium was replaced with fresh medium containing an antioxidant (Fx, Fxol, Fxac, astaxanthin, or α-tocopherol) and/or the anticancer drug cisplatin. The cells were incubated for an additional 72 h. All antioxidants were dissolved in DMSO and added to the culture medium (2-8 μM). α-Tocopherol was purchased from Wako Pure Chemical Industries, Ltd., and astaxanthin was purchased from Santa Cruz Biotechnology. The final concentration of DMSO was 0.5% (v/v), and the control culture received only DMSO. The anticancer drug cisplatin was purchased (Wako) and dissolved in water (2.5 μg/mL). Cell viability was determined by Cell Counting Kit-8 (Dojindo). After 72 h, the medium in each well of a 96-well plate was replaced...
with Phosphate-Buffered Saline (PBS). After a 1-min wash, the PBS was replaced with a solution of 5 µL of WST-8 reagent and 95 µL of cultured medium, and the cells were then incubated for 1 h at 37°C. The absorbance at 440 nm was measured using a microplate spectrophotometer (TECAN GENios). This experiment was conducted in January-March 2011.

RESULTS AND DISCUSSION

Cytotoxicity of the antioxidants: The culture media of Caco-2, Hep G2, and Neuro2a cells were supplemented with Fx, Fxol, and the other antioxidants to investigate their cytotoxic effects on cancer cells (Fig. 2-4). Dose-dependent effects on the viability of Caco-2, Hep G2, and...
Neuro2a cells were shown for Fx. At 4 μM, Fx reduced cell viability to 59% for Caco-2 and to 85% for Hep G2. At 8 μM, Fx reduced cell viability to 30% for Caco-2, 5% for Hep G2, and 69% for Neuro2a (Fig. 2). Figure 3 shows the dose-dependent effects of Fxol on the viability of Caco-2, Hep G2, and Neuro2a cells. At 4 μM, Fxol reduced cell viability to 46% for Caco-2, 85% for Hep G2, and 60% for Neuro2a. At 8 μM, Fxol reduced cell viability to 24% for Caco-2, 6% for Hep G2, and 7% for Neuro2a. Based on these results, Fx and Fxol have cytotoxic effects and are able to suppress the proliferation of cancer cells in the absence of chemotherapeutic agents. In contrast, fucoxanthin acetate, astaxanthin, and α-tocopherol had no effect on the cell viability of Hep G2 at 2-8 μM (Fig. 4).

**Effect of antioxidants on cisplatin:** To investigate the effects of Fx on chemotherapy, we studied the interaction of cisplatin and Fx (Fig. 2). Cisplatin reduced cell viability to 33% for Caco-2, 24% for Hep G2, and 25% for Neuro2a. DMSO mitigated the growth inhibition effect of cisplatin and recovered the cell viability to 47% for Caco-2, 52% for Hep G2, and 65% for Neuro2a. However, Fx did not affect the growth inhibition effect of cisplatin (at 2 μM Fx: 42% for Caco-2, 51% for Hep G2, and 69% for Neuro2a). The anti-oxidative potential of Fx did not influence cisplatin cytotoxicity. The anti-oxidative potential of Fxol and other antioxidants also did not influence the growth inhibition effect of cisplatin (Fig. 3, 4). It was shown that DMSO decreased the effect of cisplatin, while the antioxidants had no influence on the effect of the chemotherapy agent. When cisplatin is added to the culture medium, one should dissolve the carotenoids by using other solvents.

**CONCLUSION**

Our cell culture studies showed that Fx and Fxol were able to suppress the proliferation of cancer cells in a dose-dependent manner. Moreover, none of the antioxidants used in this study affected the growth inhibition potency of cisplatin. We suggest that Fx and Fxol have the potential for cancer prevention and will not interfere with anticancer drugs during chemotherapy. When 5Fu was used instead of cisplatin, it showed a comparable result (data not shown). Further studies are necessary to clarify the effect of Fx during chemotherapy.

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**REFERENCES**


