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Cancer Letters 220 (2005) 75–84
CANCER Letters
www.elsevier.com/locate/canlet

Neoxanthin and fucoxanthin induce apoptosis in PC-3 human prostate cancer cells

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Received 5 May 2004; received in revised form 6 July 2004; accepted 15 July 2004

Abstract

Neoxanthin and fucoxanthin, which have the characteristic structure of 5,6-monoepoxide and an allenic bond, were previously found to reduce the viability of human prostate cancer cells most intensively among 15 dietary carotenoids tested. In the present study, the induction of apoptosis in PC-3 cells by these two carotenoids was characterized by morphological changes, DNA fragmentation, an increased percentage of hypodiploid cells, and cleavages of caspase-3 and PARP. The ratio of apoptotic cells reached more than 30% after treatment for 48 h with 20 μM carotenoids. They reduced the expression of Bax and Bcl-2 proteins, but not Bcl-X_L. Fucoxanthin accumulated in the cells at the same level as neoxanthin. Moreover, fucoxanthinol, a deacetylated product of fucoxanthin, formed in the cells treated with fucoxanthin and reached a level comparable to that of fucoxanthin after incubation for 24 h. Treatment by fucoxanthinol alone also induced apoptosis in PC-3 cells. Thus, neoxanthin and fucoxanthin treatments were found to induce apoptosis through caspase-3 activation in PC-3 human prostate cancer cells.

Keywords: Neoxanthin; Fucoxanthin; Fucoxanthinol; Carotenoid; Apoptosis; Prostate cancer cells

1. Introduction

Much attention has been paid to the possible associations between dietary habits and the risk of prostate cancer, which is the second leading cause of cancer-related death among men in most Western countries [1]. Epidemiological studies suggest that ingestion of vitamin D, α-tocopherol, soy isoflavones, and carotenoids reduce the risk of prostate cancer [2,3]. With regard to carotenoids, the ingestion of tomato and tomato-based foods rich in lycopene and the accumulation of lycopene in plasma have been

Abbreviations: DCDDF-DA, diacetoxymethyl 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate; DMEM, Dulbecco's Modified Eagle's medium; FACS, fluorescence-activated cell sorting; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; THF, tetrahydrofuran; TUNEL, TdT-mediated dUTP nick end labeling.

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doi:10.1016/j.canlet.2004.07.048

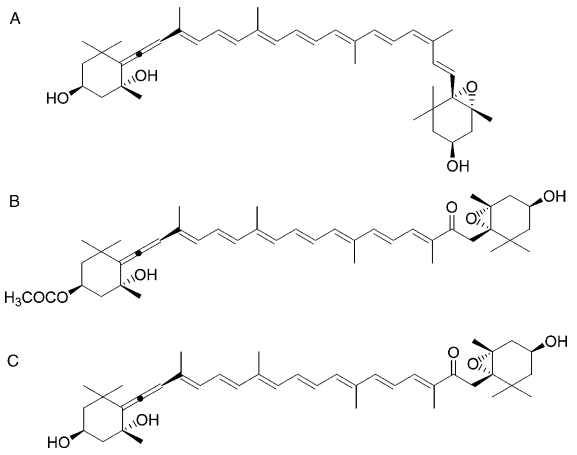

Fig. 1. Chemical structures of carotenoids. (A) 9′-*cis* neoxanthin; (B) all-*trans* fucoxanthin; (C) and all-*trans* fucoxanthinol.

reported to be significantly associated with a reduced risk of prostate cancer [4–6]. In vitro, β-carotene alone as well as the combination of lycopene and α-tocopherol inhibited the proliferation of human prostate cancer cells [7,8]. In our previous studies, we have found that various retinoids and carotenoids have an anti-proliferative effect on human prostate cancer cells of PC-3, DU 145, and LNCaP [9,10]. Neoxanthin and fucoxanthin (Fig. 1) showed the strongest anti-proliferative effect among 15 dietary carotenoids. These two carotenoids were indicated to induce apoptosis in the prostate cancer cells by in situ TdT-mediated dUTP nick end labeling (TUNEL) staining.

Since Muto et al. [11] discovered that the apoptosis induction in cervical dysplastic cells occurred via down-regulation of epidermal growth factor receptor by β-carotene, various carotenoids such as lycopene, β-cryptoxanthin, lutein, canthaxanthin, and fucoxanthin have been reported to induce apoptosis in several cancer cells [12–21]. However, the detailed mechanisms underlying apoptosis induction by carotenoids remain unknown, although apoptosis induction might be one of the important biological actions of carotenoids. Moreover, little is known concerning the relationship of apoptosis induction to cellular uptakes and to metabolic transformation of carotenoids. Recently we have shown that neoxanthin and fucoxanthin could be taken up by Caco-2 human intestinal cells [22,23] and that fucoxanthin fed to mice was circulated in blood as metabolites such as fucoxanthinol (Fig. 1) and amarouciaxanthin A [24]. Furthermore, the oxidation products of lycopene and other acyclic carotenes such as phytofluene and ζ-carotene have been suggested to induce apoptosis in HL-60 cells [15,25]. Thus, the metabolic fate of carotenoids should be studied in order to elucidate the action mechanism of carotenoids on cancer cells. In the present study, we obtained clear evidence of apoptosis induction in PC-3 human prostate cancer cells by neoxanthin and fucoxanthin, and their cellular uptake and metabolism were evaluated.

2. Materials and methods

2.1. Materials

Propidium iodide and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Hoechst 33342 was purchased from Calbiochem-Novabiochem Co. (San Diego, CA, USA). Apoptosis Screening Kit *wako* was purchased from Wako Pure Chemical Industries (Osaka, Japan). Dulbecco's Modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceutical Co. (Tokyo, Japan). Diacetoxymethyl 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (DCDDF-DA) was purchased from Molecular Probes, Inc. (Eugene, OR, USA). Fetal bovine serum was purchased from CSL Limited (Parkville, Australia). HPLC-grade tetrahydrofuran (THF) and acetonitrile were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Neoxanthin (9′-*cis* isomer) and fucoxanthin (all-*trans* isomer) were prepared as previously reported [10]. Fucoxanthinol (all-*trans* isomer) was prepared by enzymatic hydrolysis of fucoxanthin [24]. Other chemicals and solvents were of reagent grade.

2.2. Cell culture and MTT assay

PC-3 human prostate cancer cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in a DMEM supplemented with 10% heat-inactivated fetal bovine serum, 4 mM L-glutamine, and antibiotics (40 U/ml penicillin and 40 μg/ml