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The chemopreventive effect of β-cryptoxanthin from mandarin on human stomach cells (BGC-823)

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ABSTRACT

β-Cryptoxanthin, a provitaminic carotenoid, present in many fruits and vegetables, has been associated with decreased risk of chronic diseases, including cancer. The influence of β-cryptoxanthin derived from mandarin on the proliferation of the stomach tumor cell line BGC-823 was tested using MTT and cell count assay at 72 h and dose–response (from 0.01 to 20 μM). β-Cryptoxanthin suppressed the cell migration by the scratch assay. Furthermore, β-cryptoxanthin induced an accumulation of cells in the G1/G0 phase of the cell cycle (as detected by flow cytometry), which was in accordance with an increased expression of p21 and down regulations of cyclin D1 and cyclin E, detected by Western blot analysis, and β-cryptoxanthin increased the mRNA levels of retinoic acid receptor β (RARβ) with the treatment at 10 μM for 24 h. Collectively, the above findings suggest that β-cryptoxanthin could be therapeutic in the treatment of stomach cancer cell *in vitro*.

1. Introduction

Stomach cancer is the fourth most common cancer and the second leading cause of cancer-related deaths. In 2008, a total of 989,600 new stomach cancer cases emerged and an estimated 738,000 deaths were reported worldwide (Jemal et al., 2011; Kamangar, Dores, & Anderson, 2006; Parkin, Bray, Ferlay, & Pisani, 2005). The causes of stomach cancer are not yet clear but the risk is always high; quite often, these are ignored at the early stages and would not be treated right away. Major causes of this ignorance are symptoms such as common discomfort (Crew & Neugut, 2006); thus, it is very important to step up effective measures for cancer prevention and screening interventions. Diet certainly plays an important role in the gastric carcinogenesis process. The integrated role of diet, and the association between intake of fruits and vegetables and the risk of gastric cancer, have been evaluated extensively in many observational epidemiologic studies, suggesting an inverse association, particularly for the raw and allium groups of vegetables and citrus fruits with abundant carotenoids (Gonzalez et al., 2006; Nouraie et al., 2005). However, some studies are inconsistent with these associations and provide little support in this regard (Liu & Russell, 2008).

Beneficial effects of carotenoid-rich fruits and vegetables on gastric cancer have been found in numerous observational studies. In the European Prospective Investigation into Cancer and Nutrition (330 gastric cancer cases among 520,000 men and women), a protective role for citrus fruit consumption was observed for the gastric cardia cancer (Gonzalez et al., 2006). In the Netherlands Cohort Study on vegetables and fruits consumption and parallel risk of esophageal and gastric cancer subtypes, citrus fruits, including mandarins, oranges and fresh orange juice, were inversely associated with esophageal adenocarcinoma and gastric cardia adenocarcinoma risks (RR, Incidence Rate Ratio), for highest vs lowest intake: 0.55, 95% CI (confidence interval) 0.31–0.98 and 0.38, 95% CI 0.21–0.69, respectively) (Steevens, Schouten, Goldbohm, & van den Brandt, 2011). These results suggest that citrus fruits may provide protection against stomach cancer; however, the effects of major bioactive compounds in citrus fruits, including carotenoids, are yet to be elucidated in stomach carcinogenesis.

β-Cryptoxanthin (3-hydroxy-β-carotene), a provitamin A carotenoid contained in oranges and orange juice, is one of the 6 major carotenoids (β-carotene, lycopene, α-carotene, lutein and zeaxanthin) found routinely in human blood (Granado, Olmedilla, Blanco, & Rojas-Hidalgo, 1996). β-Cryptoxanthin is obtained primarily from citrus fruits (Granado et al., 1996), but is also found in pumpkins, persimmons, leafy vegetables and some yellow-coloured

Abbreviations: RARβ, retinoic acid receptor β; RAR, retinoic acid receptors; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; PI, propidium iodide; RNase A, ribonuclease A; MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CCTCC, China Center for Type Culture Collection; TLC, thin layer chromatography; HPLC–APCI–MS, high performance liquid chromatography–atmospheric pressure chemical ionisation–mass spectrometry; RXR, retinoid x receptor; PPARs, peroxisome proliferator-activated receptors.

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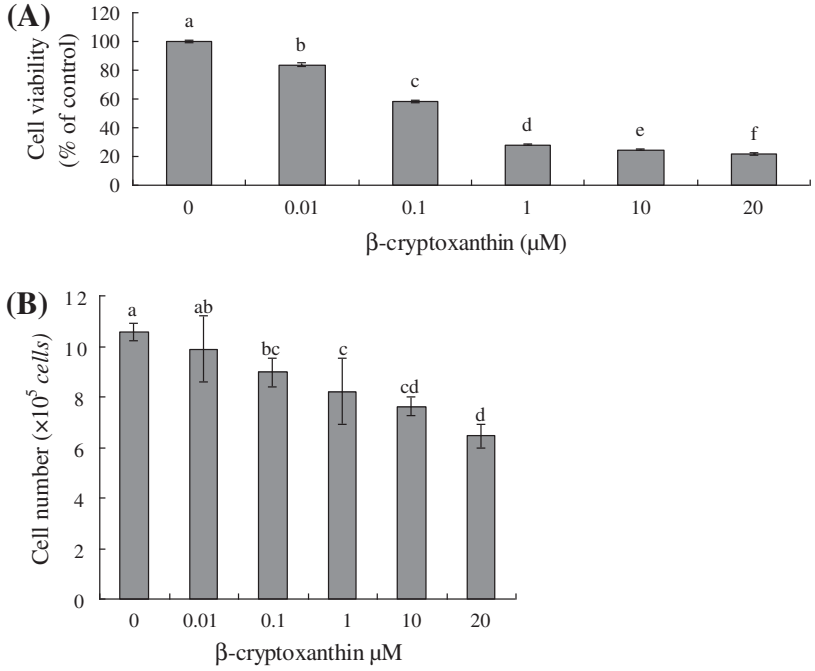

Fig. 1. The growth inhibitory effect of β-cryptoxanthin on BGC-823 cells. A, MTT assay; 4×10^3 cells were treated with the indicated concentrations of β-cryptoxanthin for 72 h, as described in Section 2; B, cell count assay; 5×10^4 cells were treated with the indicated concentration of β-cryptoxanthin for 72 h; cells were collected, and cell numbers were counted using a hemocytometer. All values are means ± SD of triplicate assays. Comparison of mean values of control and treatment cells was made using one way ANOVA, followed by Tukey's honest test for continuous variables. Different letters for given bars indicate that those values are significantly different from each other, $p < 0.05$.

animal products, such as egg yolk and butter. Like other carotenoids, β-cryptoxanthin is an antioxidant and may help to prevent free radical damage to bio-molecules, including lipids, proteins and nucleic acids (Stahl & Sies, 2005). Recent epidemiological studies revealed a reduction in risk of developing rheumatoid arthritis and lung cancer with moderate increase in β-cryptoxanthin intake. These results support the hypothesis that β-cryptoxanthin has the capacity to stimulate the differentiation of lung cancer cells and modulate the immune response through Th2 cells via retinoic acid receptors (RAR), which are elicited by retinoids, the important biologically active oxidative products from provitamin A carotenoids, and function as transcription factors and regulate gene expression (Pattison et al., 2005; Yuan, Stram, Arakawa, Lee, & Mimi, 2003). Epidemiological studies indicate that appropriate vegetable and fruit intake may be helpful in preventing cancer and cardiovascular diseases. Given the fact that vegetables and fruits contain β-cryptoxanthin, this molecule may indeed be the source of that anti-atherogenic and anti-tumor activity by acting through a RAR signalling mechanism (Matsumoto et al., 2007). However, the chemopreventive activity of β-cryptoxanthin against cancer cells has not been well investigated in stomach carcinogenesis.

In the present study, we investigated a dose-dependent antiproliferative activity of β-cryptoxanthin from mandarin fruit in an *in vitro* system, using BGC-823 stomach cancer cells; furthermore, an association was determined among different concentrations of β-cryptoxanthin on the induction of the mRNA levels of RARβ.

2. Materials and methods

2.1. Chemicals

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were supplied by HyClone. Dimethyl sulfoxide (DMSO), propidium iodide (PI) and ribonuclease A (RNase A) were purchased from Sigma–Aldrich Chemical Co. [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from GEN-VIEW SCIENTIFICINC. Trypsin (1:250) Powder was produced by Gibco BRL.

2.2. Cell culture and treatment

Stomach tumor cell line BGC-823, derived from human gastric gland adenocarcinoma, and undifferentiated with epithelial morphology, widely used in gastric cancer research (Chen, Wang, Xu, & Zhang, 2005; Du et al., 2009), was purchased from CCTCC (China Center for Type Culture Collection, Wuhan, China). It was cultured at 37 °C and 5% CO_2 in DMEM, supplemented with 10% FBS, 1% penicillin and streptomycin. During β-cryptoxanthin treatment, BGC-823 cells were transferred to a medium containing 1% FBS (Lian, Smith, Ernst, Russell, & Wang, 2007). A stock solution of β-cryptoxanthin (4 mg/ml) was made by dissolving β-cryptoxanthin in DMSO and stored at −80 °C. Aliquots from the stock solution were added to the cell culture medium at the desired working concentration, and stirred vigorously. The final DMSO concentration in the culture medium was 0.28%. The control culture received only DMSO (vehicle alone). The cell culture medium, containing β-cryptoxanthin, was changed every other day. All the procedures, including incubation with the β-cryptoxanthin, were performed under red light.

2.3. Preparation of β-cryptoxanthin

β-Cryptoxanthin was purified from mandarins (*Citrus unshiu* cv. Guoqing No. 1), which were provided by Prof. Siyi Pan (College of Food Science and Technology, Huazhong Agricultural University). Carotenoids in the mandarins were extracted as described previously by Fu et al., (2010). Briefly, initial extraction was carried out with a mixture of methanol, acetone and petroleum ether (1:1:1, v/v/v) at room temperature until colourless (Rodriguez-Amaya &