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Influence of Fucoxanthin on Proliferative Activity of Human Melanocyte Culture

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We studied the effect of algae pigment fucoxanthin on proliferative activity of melanocyte culture from human skin. Fucoxanthin in high concentrations can be cytotoxic, which was confirmed by changes in melanocyte morphology and a decrease in their proliferative activity.

Key Words: *melanocytes; fucoxanthin; drug testing; cytotoxicity; proliferative activity*

Melanocytes play a leading role in protecting the skin from the damaging effects of UV light by providing the production of pigment in the complex and multistage melanogenesis process. The search for new drugs that can affect different stages and levels of melanin synthesis regulation for suppression of hyperpigmentation and abnormal melanogenesis is in progress. Cell cultures are a unique model for testing of these products [1-3,10]. Algae pigment fucoxanthin is considered as a promising drug capable of modulating melanogenesis and producing multiple protective effects in various pathologies [6,7].

Our aim was to study the effect of different concentrations of fucoxanthin on proliferative activity of human skin melanocyte culture.

MATERIALS AND METHODS

Stock and working solutions of fucoxanthin. Fucoxanthin powder (Anhui) was dissolved in DMEM/F-12 nutrient medium (BioloT) to a concentration of 500 μM (stock solution) and sterilized by filtering through a 0.22-μ filter (Millipore). For the experiments, the stock solution of fucoxanthin was diluted with the growth medium to concentrations of 250, 50, and 5 μM.

Human skin melanocyte culture. Primary culture of human skin melanocytes (CELL Applications, Inc.) that was delivered to the laboratory in a cryopreserved state was quickly thawed at 37°C, transferred to 15-ml centrifuge tubes, Hanks solution (PanEco) was added to the cell suspension to a volume of 5-7 ml for dilution of the preserving solution, and the tubes were centrifuged (7 min, 1000 rpm, 100*g*). The supernatant was removed, the cells were resuspended in complete growth medium for melanocytes (CELL Applications, Inc.) and transferred to Petri dishes (seeding density 10^4 cells/cm^2). After attaining 80-85% confluence, the cells were harvested with Versene and 0.25% trypsin (PanEco), the cell concentration was brought to 10^4/cm^2 with complete medium, and the cells were plated on a new Petri dishes. The medium was replaced every 2 days.

Analysis of the effect of fucoxanthin on human melanocyte culture. Passage 3 melanocytes were plated to 12-well culture plates (10^4 cells/cm^2). The working solution of fucoxanthin was obtained by diluting the stock solution (500 μM) with complete growth medium at ratios of 1:1, 1:10, and 1:100. The cells were cultured in the presence of fucoxanthin for 72 h, vital observation was performed using a Cell-IQ device (CM Technologies) with photorecording every 20 min. The cells cultured in complete growth medium without fucoxanthin served as the control.

Control of proliferative activity of human melanocytes in 2D culture. To analyze changes in the number of cells in the control or in the presence of

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the test preparation, the photos obtained during 72-h culturing were analyzed. The number of cells in the field of view was automatically counted using Cell-IQ Analyzer software, and a plot describing the total number of cells in field of view as a function of time (h) was constructed. In 72 h of culturing and observation in the device, the cells were harvested from the culture surface with Versene and 0.25% trypsin. The total number of cells was counted using a Countess automatic cell counter (Invitrogen). The proliferation index (PI) was calculated by the formula: N72/N0, where N72 is the number of cells after 72-h culturing and N0 is the initial cell number.

Statistical analysis. The data were processed using Prism 8.0 GraphPad software. The hypotheses on normality of data distributions were tested using the Shapiro—Wilk and Pearson χ^2 tests. As experimental data had normal distribution, one-way ANOVA and the method of multiple comparisons were used. The results are presented as the mean±standard deviation (n=5), the significance level is assumed to be p<0.05.

RESULTS

During the first passage, the culture of human skin melanocytes was presented by small bipolar cells forming a loose reticular monolayer. The cells actively secreted dark pigment melanin; this picture corresponded to published data [4]. During passages 2 and 3, the cell morphology changed, they acquired the phenotype of long cells with multiple processes with characteristic branching of dendritic processes containing the pigment. During passage 3, the character of cell growth changed: 90% monolayer density was achieved after at least 10 days of culturing, while after 72 h, the confluence of the monolayer was 70-80% (Fig. 1, *a*).

Fucoxanthin added to the culture in a low concentration (5 μM) produced no toxic effect and did not affect the growth and viability of cells *in vitro* (Fig. 1, *b*). Cell morphology and the character of monolayer formation did not differ from the control. Cell growth decreased when fucoxanthin was added in a concentration of 50 μM; changes in cell morphology were insignificant, but the culture growth rate decreased and the

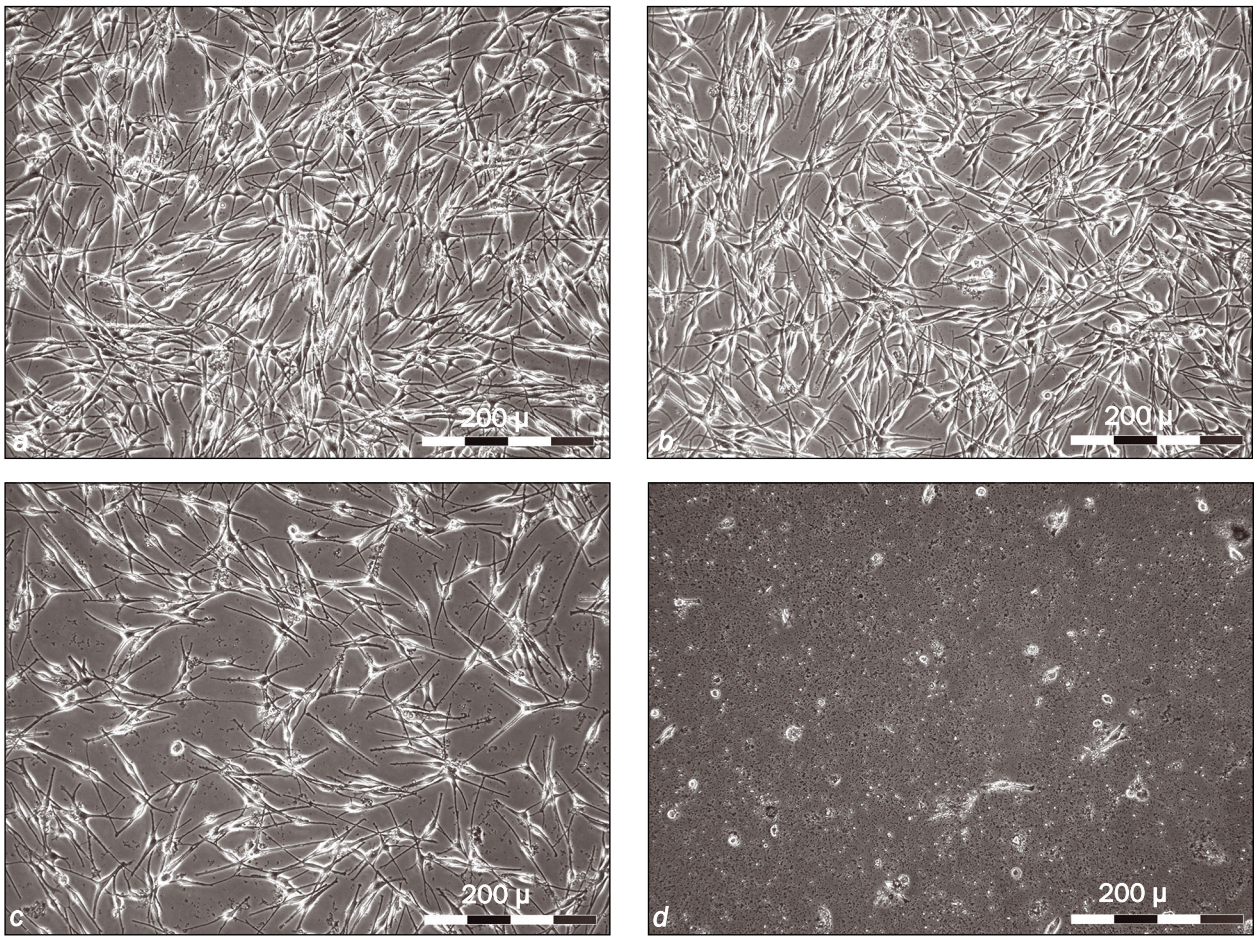

Fig. 1. The morphology and growth pattern of melanocytes after 72-h *in vitro* culturing in the absence (*a*, control) and presence of fucoxanthin in concentrations of 5 (*b*), 50 (*c*), and 250 μM (*d*).

monolayer density in 72-h culture was lower than in the control and in the presence of 5 μM fucoxanthin (Fig. 1, *c*). Addition of 250 μM fucoxanthin to the nutrient medium showed that high concentration of the preparation produced a potent toxic effect. The cells released the pigment, stopped growing, and died (Fig. 1, *d*).

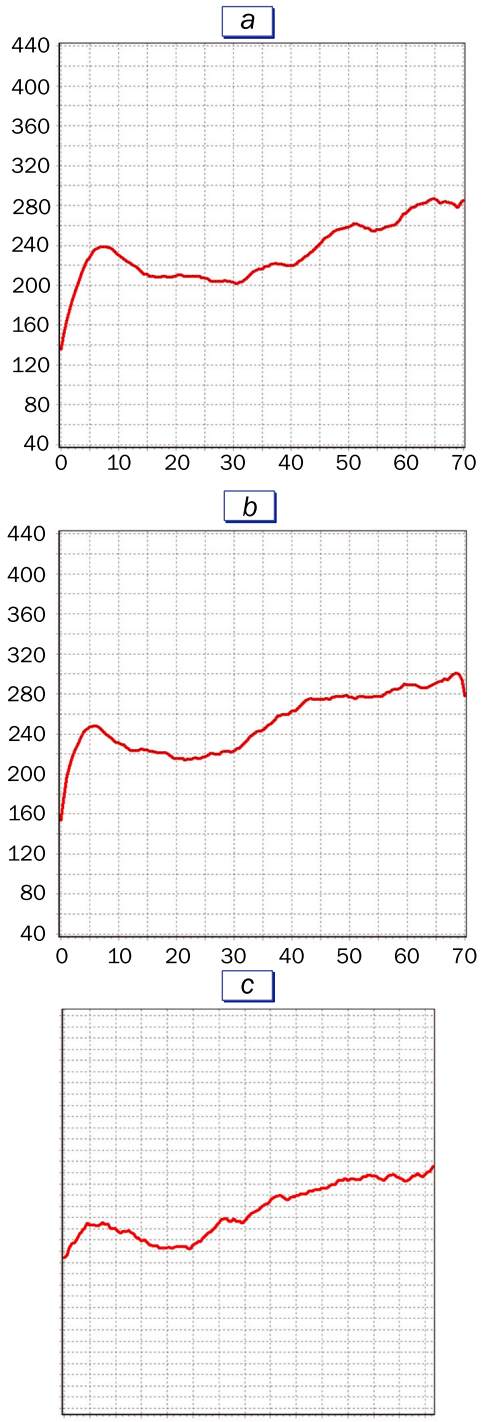

Fig. 2. Dynamics of cell number in the field of view of a Cell-IQ instrument in 2D-culture of melanocytes in the control (*a*) and in the presence of fucoxanthin in concentrations of 5 (*b*), 50 (*c*), and 250 μM (*d*).

The analysis of proliferative activity of melanocytes was performed over 72 h of culturing in the presence of different concentrations of fucoxanthin. Control cells were grown in a standard growth medium. The increase in the number of melanocytes in the field of view was observed in the control and in the presence of the lowest concentration of fucoxanthin (5 μM) with the same dynamics: the number of cells in the field of view increased by ~2 times (Fig. 2, *a*, *b*). In the presence of 50 μM fucoxanthin, the dynamics and rate of cell growth decreased (Fig. 2, *c*).

The PI of melanocyte was calculated by counting the total number of cells removed from culture plates (Fig. 3). The PI of melanocyte culture in the control and in the presence of 5 μM fucoxanthin did not significantly differ. Addition of 50 μM fucoxanthin to the melanocyte culture produced an inhibitory effect on the cell growth rate, which was consistent with the data obtained by cells counting by time-lapse microscopy. Cell death after addition of 250 μM fucoxanthin indicates its cytotoxicity in high concentrations. These data are consistent with published reports. The antiproliferative effect of the pigment in a concentration of 20 μM or higher was demonstrated in previous studies [5,8,9,11]. The use of fucoxanthin in a concentration of 5 μM did not change the growth pattern of pigment-producing human skin cells.

Fucoxanthin, isolated from seaweed exhibits unique properties. It inhibits the growth of intestinal cancer cells, breast cancer cell lines, and tumor tissue cells placed in a gel [5,8,9,11]. Moreover, fucoxanthin is considered as a promising drug for cancer prevention due to its antineoplastic effect on cells [6]. Its dose-dependent effect was demonstrated. The possibility of using fucoxanthin as a drug affecting human

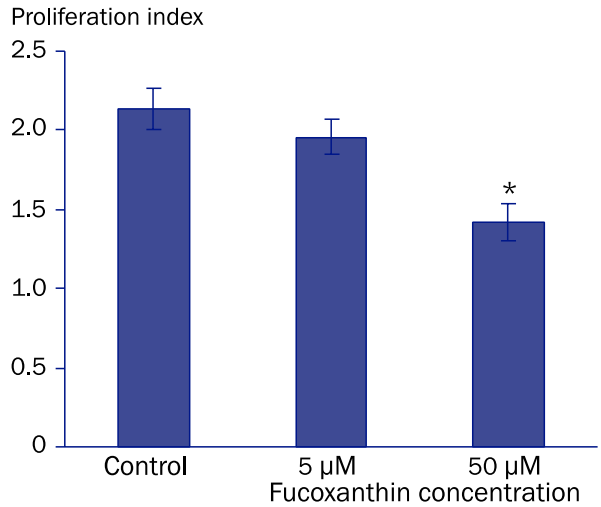

Fig. 3. PI of passage 3 melanocyte culture in the control and in the presence of fucoxanthin in concentration of 5 and 50 μM. *$p<0.05$ in comparison with the control.

skin pigment cells was proven, which makes it a promising compound for the treatment of skin pigmentation disorders, including age-related pigmentation.

The results of our experiments showed that melanocyte culture can be used as a highly sensitive method for controlling toxicity of drugs and in studies on the selection of optimal concentrations when creating cosmetic and therapeutic products. In high concentrations, fucoxanthin can be cytotoxic for normal eukaryotic cells, which is confirmed by changes in the morphology of melanocytes and a decrease in their proliferative activity.

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