Available online at www.sciencedirect.com
ScienceDirect
Toxicology in Vitro 21 (2007) 217–223
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

The influence of lycopene on the proliferation of human breast cell line (MCF-7)

F. Fornelli [a], A. Leone [b], I. Verdesca [b], F. Minervini [a,*], G. Zacheo [b]

[a] Institute of Sciences of Food Production (ISPA), Consiglio Nazionale delle Ricerche-Bari (CNR), Italy
[b] Institute of Sciences of Food Production (ISPA), Consiglio Nazionale delle Ricerche (CNR), Lecce, Italy

Received 6 April 2006; accepted 25 September 2006
Available online 6 October 2006

Abstract

Lycopene, a non-provitaminic carotenoid, present in many fruit and vegetables, such as tomatoes and their processed products, has been associated with decreased risk of chronic diseases including cancer. The influence of lycopene on the proliferation of the breast tumour cell line (MCF-7) was tested using MTT and BrdU assays at different time intervals (from 24 to 72 h) and dose–response (from 0.125 to 100 μM). The induction of Gap Junction Intercellular Communication (GJIC) was evaluated by dye-transfer assay using Lucifer Yellow on monolayer cells treated with different lycopene concentrations (from 0.125 to 5 μM) for 6 to 48 h. The Minimal Inhibitory Concentration (MIC) of lycopene was of 5 μM, after a 24 h exposure. A prolonged exposure time (72 h) induced a similar inhibitory effect. Lycopene stimulated the functionality of GJIC at concentrations of 1 μM after 24 h and this effect was dose-dependent. The induction of GJIC by lycopene was confirmed by an increased expression of connexin 43. Collectively, the above data confirm the inhibitor effects of lycopene on MCF-7 cell growth and suggest that lycopene is involved in the modulation of the gap junction intercellular communication in this cell line, as observed for other cancer cell lines.

Keywords: Lycopene; MCF-7 proliferation; GJIC; Connexin 43

1. Introduction

Lycopene, a non-provitaminic carotenoid, is present in many fruit and vegetables, such as tomatoes and their processed products, and its intake has been associated with decreased risk of chronic diseases including cancer and cardiovascular diseases (Dorgan et al., 1998).

A number of cell lines expressing estrogen receptor (ER+) are commonly found to be more sensitive to growth inhibition by retinoids than those lacking the receptor (Prakash et al., 2001). The action mode of retinoids is reported to enhance the basal expression of retinoid receptors (both RAR and RXR series) and other pathways, including the activation of protein-1-responsive genes and cell cycle specific genes (BRCA1 and BRCA2) (Prakash et al., 2001; Chalabi et al., 2004).

In addition, the lycopene or its oxidation products show ability to enhance the Gap Junction Intercellular Communication (GJIC) between preneoplastic and normal epithelial cells by increasing Connexin 43 (Cx43) expression (Bertram and Bortkiewicz, 1995; Stahl et al., 2000; Heber and Lu, 2002; Kucuk et al., 2002; Livny et al., 2002; Aust et al., 2003; Forbes et al., 2003). This activity is thought to be one of the protective mechanisms related to the cancer-preventive activity of lycopene. The Gap Junction Channels are made up of connexins and allow chemical and electric signalling or nutrient delivery (Goodenough et al., 1996). Beside, the GJCs serve to transmit growth-inhibitory signals that can inhibit the aberrant proliferation of carcinogen-initiated and fully transformed cells (Metha et al., 1986). It has been demonstrated that carotenoids increase the Cx43 expression at the message and protein levels in human and mouse fibroblasts and in suprabasal layers of human keratinocytes

* Corresponding author. Tel.: +39 (0) 80 5929360; fax: +39 (0) 80 5929374.
E-mail address: Fiorenza.minervini@ispa.cnr.it (F. Minervini).

0887-2333/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tiv.2006.09.024

grown in organotypic cultures (Hieber et al., 2000). In addition, *in vivo*, the induction of Cx43 expression by doxycycline reduces the growth rate of human cervical carcinoma cells in the "nude" mouse (King et al., 2000). These results appear to strongly suggest that agents, such as lycopene, capable of normalizing junction communication, would have cancer-preventive properties (Bertram, 2004).

The present research studied the influence of lycopene on human breast cancer cell line (MCF-7) proliferation and the capability of this carotenoid to upregulate the mRNA and protein expression of Cx43, and to enhance GJIC functionality.

2. Materials and methods

2.1. Chemicals and media

Lycopene, tetrahydrofuran (THF), MTT, RPMI 1640 medium, Dulbecco's phosphate buffered saline (PBS), L-glutamine (GLN), antibiotic–antimycotic stabilized solution (AA), heat inactivated foetal bovine serum (FBS), Trypan blue solution 0.4%, Trypsin–EDTA solution, Butylated hydroxytoluene (BHT) and Anti-Connexin-43 antibody and Anti-beta-actin in rabbits were purchased from Sigma–Aldrich (Italy). ECL Anti-rabbit IgG peroxidase-linked specie-specific $F(ab')_2$ fragment and ECL Western blotting detection reagents were purchased from Amersham Biosciences, UK. 2-propanol was purchased from Baker (Holland). ELISA BrdU colorimetric assay was purchased from Roche; Lucifer Yellow CH, 10000 MW-dextran-tetramethylrhodamine, Alexa Fluor 633-conjugated anti-rabbit IgG from Molecular Probes (Leiden, The Netherlands). SuperScript™ II Reverse Transcriptase Kit (Invitrogen), RNeasy plant mini kit (Quigen, Lajolla, CA).

2.2. Lycopene preparation

Lycopene was dissolved in THF containing 0.25% BHT, at a concentration of 10 mM and stored at −70 °C under a N_2 atmosphere. Immediately before the experiment, the lycopene was added to the cell culture medium, vigorously stirred and the obtained medium was filtered through 0.45 μm Millex-HV, (Millipore). The lycopene concentrations, ranging from 0.125 to 100 μM, were prepared using RPMI medium containing 0.025% THF–0.001% BHT (v/v). The concentration of THF never exceeded 0.5% (v/v), with the exception of the highest lycopene concentration (100 μM) in which the THF concentration was 1% (at this level did not affect the cell growth). All procedures including cell treatment were carried out under dim light.

2.3. Cell proliferation assays

2.3.1. Culture of cells

Breast cancer cell line MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, UK). The estrogen receptor characterization of MCF-7 cell line was previously reported by Minervini et al. (2005a). Cell line was routinely grown in RPMI-1640 medium containing 10% FBS, 1% GLN and 1% AA in 25 cm^2 plastic flasks (Iwaki, Japan) at 37 °C in a 5% CO_2 humidified atmosphere. Cells were passaged at 70–80% confluence, about twice a week by trypsinization. For each experiment, the MCF-7 cells were seeded in 96 multiwell plates (Iwaki, Japan) at 10^5 cell/well in 200 μl of RPMI medium supplemented with FBS, GLN and AA and cells were allowed to attach for 24 h. After this time the culture medium was changed and each concentration of lycopene, within a range from 0.125 to 100 μM, was tested in triplicate wells. The controls (with and without 0.025% THF–0.001% BHT) were included on each plate. The cells were then incubated for various periods of time with daily medium replacement (the half-life of lycopene is calculated to be about 12–20 h) (Levy et al., 1995). Cell viability was assessed by Trypan blue dye exclusion in order to evaluate the cytotoxic effect induced by lycopene treatment.

2.3.2. MTT colorimetric assay

After 24, 48 and 72 h of incubation, the cell proliferation was measured using the MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] test, as previously described (Minervini et al., 2005b). Absorbance was measured at 580 nm in a plate reader (ELISA Reader Multiskan MS Plus MK II Labsysten, Finland). Dose–response curves (0.125, 0.5, 1, 5, 10, 20, 50 and 100 μM lycopene) were plotted against the mean values calculated as percentages of untreated cells (control with THF–BHT).

2.3.3. BrdU uptake colorimetric assay

The 5-bromo-2′-deoxyuridine (BrdU) uptake colorimetric assay was used to determine cell proliferation by BrdU incorporation into cellular DNA. The assay was performed according to the manufacturer's instruction, as in Minervini et al. (2005b). Briefly, after exposure of cell cultures to different concentrations of lycopene (0.125, 0.5, 1, 5, 10, 20, 50 and 100 μM lycopene)for 24 and 48 h, cells were incubated for 4 h at 37 °C with 10 μM BrdU solution. A peroxidase-conjugated mouse monoclonal antiBrdU-POD antibody was added. Immune complex formation was revealed measuring the absorbance of the substrate reaction (tetramethylbenzydine) at 450 nm using an ELISA Reader. Absorbance values were directly correlated with the amount of DNA synthesis and thereby with the number of proliferating cells in the respective microcultures. Mean absorbance values at each lycopene concentration were compared to mean control values and were expressed as the percent of control response (cells treated with THF–BHT).

2.4. Gap-junction communication

2.4.1. Estimation of GJIC by dye-transfer assay

To verify the effect of lycopene on GJIC, the gap junction communications were measured by the fluorescent dye