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Antigenotoxicity and antimutagenicity of lycopene in HepG2 cell line evaluated by the comet assay and micronucleus test

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Abstract

Epidemiological studies have provided evidence that high consumption of tomatoes effectively reduces the risk of reactive oxygen species (ROS)-mediated diseases such as cancer. Tomatoes are rich sources of lycopene, a potent singlet oxygen-quenching carotenoid. In addition to its antioxidant properties, lycopene shows an array of biological effects including antimutagenic and anticarcinogenic activities. In the present study, the chemopreventive action of lycopene was examined on DNA damage and clastogenic or aneugenic effects of H_2O_2 and *n*-nitrosodiethylamine (DEN) in the metabolically competent human hepatoma cell line (HepG2 cells). Lycopene at concentrations of 10, 25, and 50 μM, was tested under three protocols: before, simultaneously, and after treatment with the mutagen, using the comet and micronucleus assays. Lycopene significantly reduced the genotoxicity and mutagenicity of H_2O_2 in all of the conditions tested. For DEN, significant reductions of primary DNA damage (comet assay) were detected when the carotenoid (all of the doses) was added in the cell culture medium before or simultaneously with the mutagen. In the micronucleus test, the protective effect of lycopene was observed only when added prior to DEN treatment. In conclusion, our results suggest that lycopene is a suitable agent for preventing chemically-induced DNA and chromosome damage.

Keywords: Lycopene; DNA damage; Antigenotoxicity; Antimutagenicity; HepG2 cells

1. Introduction

Since the hypothesis by Peto et al. (1981) that β-carotene might reduce the incidence of cancer, several epidemiological studies have shown that cancer risk is inversely related to the consumption of foods rich in carotenoids. Lycopene, a carotenoid mainly found in tomatoes, is an acyclic isomer of β-carotene, but with no vitamin A activity (Rao and Agarwal, 2000). It is a natural pigment synthesized by plants and microorganism (Gruenwald et al., 2003), and one of the most potent antioxidants (Di Mascio et al., 1989).

In addition to its antioxidant properties, lycopene has an array of biological effects including cardioprotective, anti-inflammatory, antimutagenic and anticarcinogenic activities (Bhuvaneswari and Nagini, 2005). The mechanisms underlying the inhibitory effects of lycopene on carcinogenesis and mutagenesis could involve radical oxygen species (ROS) scavenging, up-regulation of detoxification (Astorg et al., 1997), interference with cell proliferation (Pastori et al., 1998), induction of gap-junctional communication (Zhang et al., 1991), inhibition of cell cycle progression, and modulation of signal transduction pathways (Bhuvaneswari and Nagini, 2005).

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Different cell types have been used to determine genotoxicity and antigenotoxicity of some natural compounds (Yusuf et al., 2000). Since many carcinogens require metabolic activation to react with DNA, the use of cells that possess endogenous biotransforming activity can reduce some of the problems associated with the use of exogenous activation mixtures such as S9 mix (Valentin-Severin et al., 2003). Human hepatoma cell lines, the most promising being the HepG2 cell line, appear to be a practical alternative for assessing genotoxicity or antigenotoxicity (Salvadori et al., 1993). HepG2 cells are easy to handle and contain several enzymes responsible for the activation of various xenobiotics (Diamond et al., 1980; Sassa et al., 1989).

Among short-term mutagenicity/genotoxicity assays, the micronucleus (MN) and the comet assays have been widely used for identifying chemopreventive agents. These two tests are sensitive, easy to perform, and can be carried out with various cell lines, including HepG2 (Salvadori et al., 1993; Uhl et al., 2000). The difference is basically due to variations in the type of DNA alterations detected by these two assays: the MN test detects irreparable lesions that manifest as chromosome aberrations and/or aneugenic effects while the comet assay detects primary DNA lesions. Thus, these assays were used to investigate the chemopreventive activity of lycopene on DNA damage induced in HepG2 cells by a direct (hydrogen peroxide) and an indirect (*n*-nitrosodiethylamine) mutagen.

2. Materials and methods

2.1. Cells and culture conditions

HepG2 cells were grown as monolayer cultures in DMEM medium (Cultilab, Brazil) supplemented with 15% fetal calf serum (Cultilab, Brazil) and antibiotics, penicillin (100 U/mL) and streptomycin (0.1 mg/mL). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Confluent cells were trypsinized for 10 min. Next, 10 mL complete medium was added and cells were centrifuged at 180*g* for 5 min. Cell suspensions at densities of 0.25×10^6 cells per well or 1×10^6 cells per plate were seeded in 24-well plates and in 94-mm plastic dishes for the comet assay and micronuclei assay, respectively. Subcultures were performed on the day before the experiments. The viability of the cells was checked in all experiments at each concentration of lycopene tested. In brief, a freshly prepared solution of 10 μL Tripan blue (0.05%) in distilled water was mixed to 10 μL of each cellular suspension during 5 min, spread onto a microscope slide and covered with a coverslip. Non-viable cells appear blue-stained. At least 200 cells were counted per-treatment.

2.2. Chemicals

Lycopene (>96%), obtained from Lycored Natural Products (Israel), was dissolved into the culture medium plus Tween 80 (980 μL culture medium +20 μL Tween) at concentrations of 10, 25, and 50 μM, just before use. The mutagens, *n*-nitrosodiethylamine (DEN) (Sigma, USA) and hydrogen peroxide (Merck, USA), were dissolved into DMEM medium just before treatment. The doses of mutagens and lycopene were established in previous pilot studies conducted in our laboratory in order to evaluate antigenotoxicity and/or antimutagenicity of lycopene with accuracy.

2.3. Cell treatment for the comet assay

Each protocol was performed in triplicate to ensure reproducibility.

Pre-treatment: twenty-four hours after seeding, the medium was removed and cells were treated for 1 h with lycopene at one of the three concentrations (10, 25, or 50 μM). Cells were then washed with PBS and treated with DEN (5 μg/mL) or H_2O_2 (0.1 mM) for 1 h or 10 min, respectively, since DEN needs a longer exposure time to exert genotoxicity. After treatments, the cells were washed twice with PBS, trypsinized, centrifuged at 180*g* for 3 min and resuspended into 100 μL of fresh medium.

Simultaneous treatment: twenty-four hours after seeding, the medium was removed, and cells were simultaneously treated with lycopene (10, 25, or 50 μM) and with DEN (5 μg/mL) or H_2O_2 (0.1 mM) for 1 h or 10 min, respectively. Then, cells were washed twice with PBS, trypsinized, centrifuged at 180*g* for 3 min and resuspended into 100 μL of fresh medium.

Post-treatment: twenty-four hours after seeding, the medium was removed and cells treated with DEN (5 μg/mL) or H_2O_2 (0.1 mM) for 1 h or 10 min, respectively. Cells were then washed with PBS, and lycopene (10, 25, or 50 μM, in 10 mL medium) was added for 1 h at 37 °C. After this period, cells were washed twice with PBS, trypsinized, centrifuged at 180*g* for 3 min, and resuspended into 100 μL of fresh medium.

The comet assay was performed by following the protocol of Tice et al. (2000). Briefly, a volume of 10 μl of cells was mixed with 120 μL of 0.5% low-melting point agarose at 37 °C, layered onto a precoated slide with 1.5% regular agarose and covered with a coverslip. After brief agarose solidification at 4 °C, the coverslip was removed and the slides immersed into a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl buffer, pH 10, 1% sodium sarcosinate with 1% Triton X-100 and 10% DMSO) for at least 1 h. After lysis, slides were exposed to alkaline buffer (pH >13) for 20 min and subjected to electrophoresis to 20 min (25 V, 300 mA). Then, the slides were neutralized with 0.4 M Tris–HCl buffer (pH 7.5), fixed in absolute ethanol and stored at 4 °C until analysis. Fifty randomly selected cells per-treatment (well) were examined at 400× magnification, on a fluorescence microscope by using an automated image analysis system (Comet Assay II, Perceptive Instruments, UK). The parameter selected as indicator of DNA damage was tail moment (product