Biochemical and Biophysical Research Communications 434 (2013) 479–485

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Evaluating the effect of lycopene from *Lycopersicum esculentum* on apoptosis during NDEA induced hepatocarcinogenesis

Prachi Gupta, Mohinder Pal Bansal, Ashwani Koul *

Department of Biophysics, Basic Medical Sciences Block, Panjab University, Chandigarh 160014, India

ARTICLE INFO

Article history:
Received 22 March 2013
Available online 11 April 2013

Keywords:
Lycopene
N-nitrosodiethylamine
Apoptosis
Redox environment
Hepatocarcinogenesis

ABSTRACT

The present study was aimed to examine the influence of lycopene from tomatoes (LycT) on apoptosis in N-nitrosodiethylamine (NDEA) induced hepatocarcinogenesis. Female Balb/c mice were randomly divided into four groups i.e. Control, NDEA, LycT and LycT + NDEA. Hepatic tissue from NDEA treated mice exhibited enhanced expression of anti-apoptotic gene bcl-2 and decreased expression of pro-apoptotic genes caspase 3, 9 and p53 when compared to control group. LycT intervention to NDEA challenged mice exhibited enhanced expression of caspase 3, 9 and p53 and decreased expression of bcl-2 when compared with NDEA treated animals. Enhanced DNA damage was revealed in NDEA and LycT + NDEA groups as revealed by comet assay. However, TUNEL assay indicated enhanced apoptosis in LycT + NDEA group when compared to NDEA group. Hepatic tissue of NDEA treated mice showed persistently high lipid peroxidation levels and glutathione redox ratio during the process of hepatocarcinogenesis. The observed enhanced apoptosis in LycT + NDEA group may be attributed to its differential effects on apoptosis associated genes and its ability to act as a pro-oxidant. These findings provide a rational mechanistic insight into the growth-inhibitory effects of lycopene against hepatic cancer.

1. Introduction

Exposure to chemical carcinogens leads to various biochemical and genetic alterations in the cell. N-nitrosodiethylamine (NDEA), a known environmental hepatic carcinogen, has been used as an initiator in several hepatic cancer models [1]. Cytochrome P450 (CYP) mediated NDEA metabolism generates reactive oxygen species (ROS) and other free radicals, which may be responsible for its hepatocarcinogenic effects [2]. Hepatocellular carcinoma (HCC) is ranked among the most common cancer in the world, and is one of the leading causes of cancer associated deaths [3]. In fact, lack of curative therapies and their associated adverse effects demands new strategies to address the issue of liver cancer with lesser or no side effects. One such strategy might include incorporating large amounts of natural compounds thought to have chemopreventive effects against cancer in diet or to ingest a dietary supplement containing an efficacious quantity of such a compound. Therefore, research towards preparation of phytochemical formulations and use of natural dietary supplements for combating carcinogenesis has gained momentum [1,4,5].

Lycopene, a polyunsaturated hydrocarbon, is the most abundant carotenoid, present in tomatoes (*Lycopersicum* esculentum) with concentrations ranging from 0.9 to 4.2 mg/100 g [6]. It is a potent antioxidant with highest singlet oxygen quenching efficiency and may modulate mutagenesis and carcinogenesis [7,8]. For these reasons several epidemiological and small scale studies have given lycopene, a privileged status in natural product research and have associated it with decreased risk of cancer and cardiovascular diseases [9,10]. Therefore, unraveling the pathways intervened by lycopene during tumorigenesis could provide a rational approach for using it as a chemopreventive agent. Apoptosis is arguably one of the most potent defenses against cancer because evasion of apoptosis is one of the hallmarks for the promotion and progression of cancer as well as treatment resistance [11,12]. Therefore, considering the importance of apoptosis in cancer, the present study was designed to determine the involvement of lycopene in influencing the apoptotic pathway while counteracting NDEA induced hepatocarcinogenesis.

2. Material and methods

2.1. Materials

Lycopene (LycT) was extracted from tomatoes as described previously [13]. Oligonucleotides were synthesized by Sigma–Aldrich (USA). Antibodies against bcl-2, caspase 3, caspase 9, p53, β-actin and peroxidise-conjugated anti-rabbit secondary antibody were purchased from Santa Cruz Biotechnology, Santa Cruz, CA (USA).

* Corresponding author.
E-mail addresses: drashwanikoul@yahoo.co.in, ashwanik@pu.ac.in (A. Koul).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.099

2.2. Animal model and experiment conditions

Female Balb/c mice, 25–30 g each were randomly divided into four groups. Group I (Control) animals received 0.1 ml olive oil (vehicle) orally thrice a week for 24 weeks. Group II (NDEA) animals received a cumulative dose of 200 mg NDEA/kg body weight intraperitoneally in 8 weeks as previously described [1]. Group III (LycT) animals were administered LycT orally at a dosage of 5 mg/kg body weight in olive oil thrice a week throughout the experiment (24 weeks). The first dose of NDEA was commenced after 2 weeks of pre- treatment with LycT in group IV (LycT + NDEA). The experimental animal studies were approved by the ethical committee, Panjab University, Chandigarh and conducted adhering to the Indian National Science Academy Guidelines (New Delhi, India) for the use and care of experimental animals. The treatment schedule carried out in the study is shown in Fig. 1.

2.3. Apoptosis analysis

2.3.1. TUNEL assay

Apoptosis was demonstrated after 24 weeks of treatment in different groups by the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) method using a standard procedure included in TACS.XL-DAB detection kit from TREVIGEN (Gaithersburg). Incorporation of brominated nucleotides mixture (B-dNTP) by terminal deoxynucleotidyl transferase enzyme (TdT) at the site of DNA fragments are detected using highly specific and sensitive biotinylated anti-BrdU antibody. It generates dark brown staining in the apoptotic cells that is easily visualized against green counterstain. Apoptotic index was determined by counting the number of TUNEL (+ve) cells over the total number of cells in the selected liver tissue section (300 cells), and apoptotic index was expressed based on this percentage.

2.3.2. Comet assay

After 24 weeks single cell gel electrophoresis (SCGE) was performed as described by Tice et al. [14]. Briefly, hepatocytes were prepared by collagenase perfusion as previously described [15]. Comet assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of the nucleoid under the influence of an electric field, whereas undamaged DNA migrates slower and remains within nucleoid. Approximately 200 cells were examined per slide for comet images and scored blindly by two observers.

2.3.3. mRNA expression analysis by RT-PCR

Total RNA was isolated from the hepatic tissue using Tri-Reagent (Molecular Research Centre, Inc., Cincinnati, Ohio) after 24 weeks of treatment. For the RT-PCR analysis primers for the following genes: bcl-2, caspase 3, caspase 9, p53 and β-actin were designed from the sequence data with the help of software 'Gene Runner'. Primers designed for different genes are mentioned in Table 1. mRNA expression was demonstrated by RT-PCR method using the standard procedure described in Superscript III one step RT-PCR kit from Invitrogen (California). The final PCR products formed were analyzed on 1.5% agarose gel electrophoresis and densitometric analysis of the bands was done by Image J software (NH, USA).

2.3.4. Quantitation of protein expression by ELISA

10% (w/v) liver homogenates were prepared in 50 mM Tris–HCl (pH 7.4) under ice-cold conditions and treated with triton-X 100 and phenylmethylsulfonyl fluoride, a protease inhibitor. Homogenates were then centrifuged at 11,800 g for 30 min. PMF thus

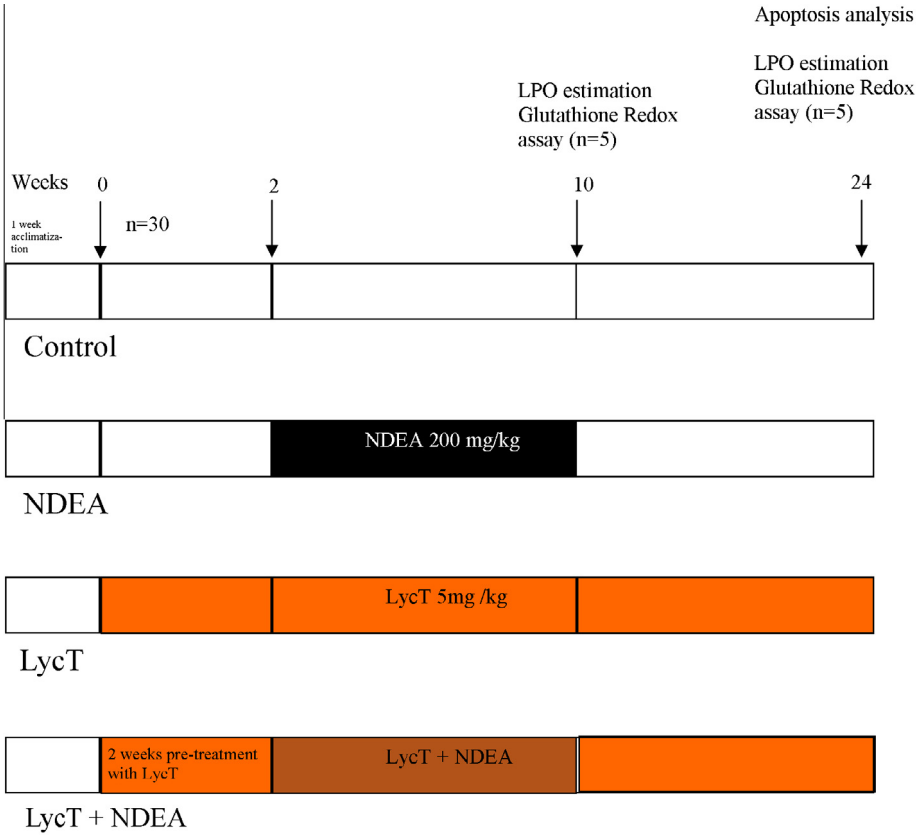

Fig. 1. Treatment protocol in different groups of animals.