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The Effect of Lycopene on Oxidative DNA Damage in Human Lymphocytes

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Introduction

Reactive oxygen species (ROS) react with cellular components, causing oxidative damage to critical cellular biomolecules as lipids, proteins, and DNA. There is strong evidence that oxidative damage may play a significant role in the causation of several chronic diseases including arteriosclerosis, cataract, age-related macular degeneration, multiple sclerosis, and cancer[1,2].

Oxidative DNA damage may involve the breakage in single and double-strands, base modifications, fragmentation of deoxyribose, formation of DNA-protein cross-links as well as abasic sites[3,4]. DNA strands breaks in eukaryotic cells can be detected by single cell gell electrophoresis (comet assay), with and without the addition of the repair enzymes endonuclease-III (Endo III), formamidopyrimidine N-glycosylase (Fpg), to characterize DNA lesions. Fpg initiates the repair of oxidized bases by excising them and cutting the sugar-phosphate backbone of DNA molecule. Thus additional strand breaks are induced at the location of oxidized base, causing DNA relaxation and migration. The detection of Fpg-sensitive DNA lesions revealed the presence of oxidized purine bases[5-7]. Comet assay, which is a simple, sensitive, rapid and versatile assay, is commonly used for the assessment of protective effects of antioxidants on DNA damage in intervention studies[8].

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Antioxidants are protective agents that inactivate reactive oxygen species and therefore significantly delay or prevent oxidative damage. Lycopene, a carotenoid compound, an acyclic isomer of b-carotene, widely present in tomatoes, processed tomato products, and other fruits, play an important role in human diet. It has been shown that lycopene could act as an antioxidant and protect cellular macromolecules against oxidative stress. Dietary intake of food containing lycopene has been estimated to contribute to the inhibition of the diseases believed to be initiated by reactive oxygen species.

In the present study, the antioxidant capacity of lycopene was determined using the trolox equivalent antioxidant capacity (TEAC) assay and the modulating effects of lycopene against the oxidative DNA damage induced by H_2O_2 in human lymphocytes were investigated by the standard single cell gel electrophoresis (comet assay) and Fpg-modified comet assay

Materials and Methods

Chemicals and reagents

The compound lycopene (Redivivo) used in the experiments was from DSM/Roche-Turkey; the purity of the compound was 95 %. The other chemicals used in the comet assay and TEAC assay were purchased from the following suppliers. Normal melting agarose (NMA) and low melting agarose (LMA) were from Boehringer Mannheim (Germany); sodium chloride (NaCL), potassium chloride (KCL), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), and hydrochloric acid (HCL) were from Merck Chemicals (Darmstadt, Germany); heparine, dimethyl-sulfoxide (DMSO), ethidium bromide (EtBr), Triton X-100, phosphate buffered saline (PBS) tablets, bovine serum albumin,, formamidopyrimidine-DNA glycosylase (Fpg), potassium peroxodisulphate, and 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid (ABTS) were from Sigma (St. Louis, MO); ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA-Na_2), N-lauroyl sarcosinate, and Tris were from ICN Biochemicals (Aurora, OH, USA), HPLC grade ethanol was from Fluka Chemie AG., Histopaque-1077 was

from Lymphoprep™ (Axis-Shield PoC AS, Oslo, Norway) and 6-hydoxy-2,5,7,8-tetramethylcroman-2-carboxylic acid (trolox) was from Calbiochem/ Roche.

Trolox equivalent antioxidant capacity assay (TEAC)

The TEAC method is one of the most often used methods for the determination of total antioxidant capacity.[9-11] It is based on the neutralization of the radical cation formed by a single-electron oxidation of a synthetic ABTS chromofore to a strongly absorbing $ABTS^{\cdot+}$ radical. The radical reacts quickly with electron/hydrogen donors to form colorless ABTS. A decrease of the $ABTS^{\cdot+}$ concentration is linearly dependent on the antioxidant concentration including trolox as a calibrating standard. A mixture of 7 mM ABTS and 2.45 mM potassium persulfate (1:1, v/v) was incubated for 12 h at room temperature in the dark to form $ABTS^{\cdot+}$ radical. The working solution was diluted with ethanol to give an absorbance of around 0.8 at 734 nm. After preparing trolox standards and lycopene samples, they were mixed with equal volumes of ABTS solution and the decrease in absorbance was measured at 734 nm after 2 min. The trolox concentration is proportional to the change in absorbance of the ABTS solution. A standard calibration curve was constructed for trolox at 0.5 - 20 μM concentrations. 500 μL of lycopene solutions at concentrations of 0.5 - 20 μM in ethanol were mixed with 500 μL of ABTS. Samples were assayed in three replicates. The absorbance of the resulting oxidized solution was compared to that of the calibrated trolox standard.

Single cell gel electrophoresis (comet assay) and Fpg-modified comet assay

For each experiment, 5 mL heparinized (50 units/mol sodium heparin) whole blood was collected by venepuncture from a healthy 28-year-old non-smoker female donor not exposed to radiation or drugs. The replicate experiments were carried out with blood samples from the same donor collected at different time intervals.

Lymphocytes were isolated by Ficoll-Hypaque density gradient and washed with PBS[12]. Cell concentrations were adjusted to approximately 2 x 10^5/mL in the buffer. The cells were suspended in a total volume of 1 mL and each reaction contained 50 μL suspension (» 10^4 cells), varying micro liter amounts of lycopene (L) dissolved in DMSO at the concentrations

of 0.5-14 μM. The DMSO volume of the samples never exceeded 1 % in the comet assay. The cells were incubated for 30 min h at 37 ^{0}C in an incubator together with untreated control samples. Control incubations contained the same concentrations of DMSO. After incubation the lymphocytes were harvested by centrifugation at 800 xg for 3 min at 4 ^{0}C. Oxidative damage was introduced by replacing the medium with PBS containing at 0.05 mM concentration of H_2O_2, and the treatment was applied for 5 min on ice. Then the cells were harvested by centrifugation at 800 xg for 3 min at 4 ^{0}C. Again the cells were harvested by centrifugation at 800 xg for 3 min at 4 ^{0}C after washing with PBS. The lymphocytes were suspended in 75 μL of low melting point agarose (LMA) for embedding on slides. Cells were checked for viability by trypan blue exclusion test. This method is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue. Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemacytometer.

The basic alkaline technique of Singh *et al*[13], as further described by Collins *et al*[7], was followed. Each microscopic slides had been each covered with 1% NMA at about 45 ^{0}C in Ca^{2+}-and Mg^{2+}-free PBS before the experiment. This layer was used to promote the attachment of the second layer. For the second layer, around 10000 treated or control cells mixed with 75 μL of 0.5% LMA were rapidly pipetted onto this slide, spread using a cover slip, and maintained on an ice-cold flat tray for 5 min to solidify. After removal of the cover slip, the slides were immersed in cold lysing solution, (2.5 M NaCL, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, pH 10), with 1% Triton-X 100 and 10% DMSO added just before use for a minimum of 60 min at 4^{0}C but the cells treated with H_2O_2 and embedded on slides were immersed in other cold lysing solution. The alkaline comet assay using formamidopyrimidine-DNA glycosylase (Fpg), lesion-specific enzyme was used to detect oxidized purines as a result of oxidative stress-induced DNA damage as described with some modifications[5]. The cell-agarose suspension slides were prepared as described above for the standard comet assay. After lysing, for the examination of levels of oxidized purine bases in lymphocytes, the slides were washed with enzyme buffer (40 mM HEPES, 100 mM KCL, 0.5 mM EDTA and 0.2 mg/mL bovine serum albumin) at room temperature and then incubated with Fpg protein (1 mg/mL in enzyme buffer for 30 min at 37 ^{0}C in an incubator and washed with the neutralizing

solution. Then the slides were applied to electrophoresis.

The slides were removed from the lysing solution, drained, and placed side by side avoiding space and with the agarose ends facing each other nearest the anode in a horizontal gel electrophoresis tank. The tank was filled with fresh electrophoresis solution (1 mM Na_2EDTA and 300 mM NaOH, pH 13) to a level approximately 0.25 cm above the slides. Before electrophoresis, the slides were left in the solution for 20 min to allow the unwinding of the DNA and expression of alkali labile damage. Electrophoresis was conducted at a low temperature (4 ^{0}C) for 20 min using 24 V and adjusting the current to 300 mA by raising or lowering the buffer level and using a compact power supply (Power Pack P 25 Biometra Analytic GmbH). All of these steps were carried out under dimmed light (tank was covered with a black cloth) to prevent the occurrence of additional DNA damage. After electrophoresis, the slides were taken out of the tank. Tris buffer (0.4 M Tris, pH 7.5) was added dropwise gently to neutralize the excess alkali and the slides were allowed to sit for 15 min., and then the slides were allowed to sit 5 min in 50%, 75%, and 95% alcohol, successively.

The dried microscope slides were stained with EtBr (20 μg/ml in distilled water, 60 μl/slide), The slides were covered with a cover slip, placed in a humidified airtight container to prevent drying of the gel, and analyzed within 3-4 h.

The microscope was connected to a charge-coupled device camera and a personal computer-based analysis system (Comet Analysis Software, version 3.0, Kinetic Imaging Ltd., Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of the DNA fragments in the agarose gel. In order to visualize DNA damage, the slides were examined at 100x. DNA damage was expressed as tail intensity, tail migration, and tail moment. 100 cells from each of two replicate slides were assayed. Analysis was performed by one slide reader, thus minimizing variability due to subjective scoring.

Statistical analysis

Statistical analysis was performed by the computer program SPSS for Windows 15.0. Differences between the means of data were compared by the one way variance analysis (ANOVA) test and post hoc analysis of

group differences was performed by least significant difference (LSD) test. The Kruskal-Wallis *H* test was used in comparing parameters displaying abnormal distribution between groups. The results were given as the mean ± standard deviation and *the P* values of less than 0.05 were considered as statistically significant.

Results and Discussion

Cell viability, as tested using trypan blue dye exclusion of each treated group, was more than 90 %.

Lycopene demonstrated significant antioxidant capacity within the concentrations of 0.5-20 μM in a dose dependent with respect to ABTS radical solution diluted with ethanol giving an absorbance of around 0.8 at 734 nm. At the same concentrations, the antioxidant activity of lycopene was found to be significantly higher than the antioxidant activity of trolox (Figure 1). Trolox equivalent values of lycopene using the standard calibration curve of trolox was given in Table I. The trolox equivalent antioxidant capacity of lycopene increased in a dose dependent.

The effects of lycopene on DNA strand breakage in human lymphocytes with or without H_2O_2 are shown in Table II. According to the data from three separate experiments, no additional DNA strand breakage in human lymphocytes was observed at the concentrations of 0.5, 1, 2, and 8 μM lycopene. However at all concentrations studied above 12 μM, lycopene alone seemed to induce DNA damage significantly when compared with the negative control (1% DMSO). The numbers of damaged cells were significantly reduced when lymphocytes were incubated with 0.05 mM H_2O_2 and lycopene within the concentration range of 0.5 - 14 μM as seen in Table II. Even above the concentration of 12 μM that caused DNA damage alone, lycopene seemed to have a protective effect against H_2O_2-induced DNA breakage. In addition at the concentration of 14 μM, 2 μM, and 12 μM, for tail intensity, tail moment, and tail migration, respectively, lycopene had the most DNA damage reducing effect against H_2O_2-induced DNA damage.

As shown in Table III, DNA damage expressed as tail intensity, tail moment, and tail migration in the Fpg and H_2O_2-treated lymphocytes

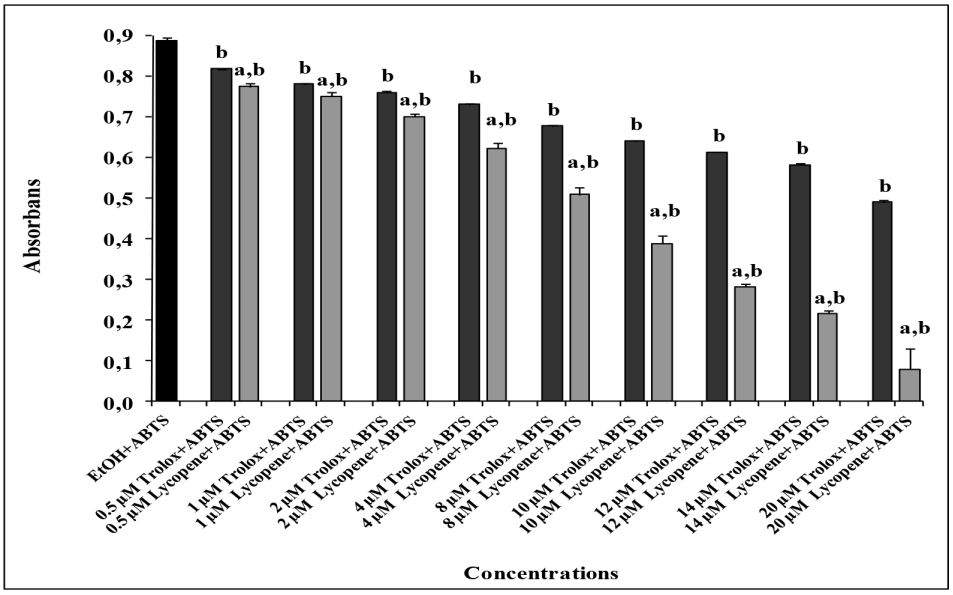

Figure 1

The antioxidant activity of lycopene and trolox on ABTS. Results were given as the mean ± standard deviation. [a]p<0.05, lycopene compared to trolox (0.5, 1, 2, 4, 8, 10, 12, 14, and 20 µM); [b]p<0.05, lycopene or trolox compared to EtOH+ABTS.

TABLE I

The trolox equivalent antioxidant capacity of lycopene*

	TE (µM)
0.5 µM Lycopene	3.58
1 µM Lycopene	4.19
2 µM Lycopene	5.46
4 µM Lycopene	7.44
8 µM Lycopene	10.39
10 µM Lycopene	13.45
12 µM Lycopene	16.19
14 µM Lycopene	17.87
20 µM Lycopene	21.39

*Trolox equivalent value (TE) was calculated according to the trolox standart curve.

TABLE II
The effects of lycopene (L) on H_2O_2-induced oxidative DNA damage in human lymphocytes*

	Tail intensity	**Tail moment**	**Tail migration**
(-) Control (DMSO)	3.20±6.09	0.02±0.05	0.09±0.19
(+) Control (H_2O_2)	52.68±36.65 [a]	0.75±0.58 [a]	1.93±1.17 [a]
0.5 μM L	1.01±3.00 [b]	0.01±0.02 [b]	0.05±0.12 [b]
1 μM L	1.36±5.30 [b]	0.01±0.07 [b]	0.06±0,17 [b]
2 μM L	2.70±5.15 [b]	0.02±0.03 [b]	0.09±0.18 [b]
4 μM L	3.35±7.42 [b]	0.02±0.06 [b]	0.14±0.28 [b]
8 μM L	4.62±9.60 [b]	0.04±0.11 [b]	0.25±0.43 [b]
10 μM L	9.07±15.65 [a,b]	0.09±0.20 [b]	0.38±0.56 [b]
12 μM L	34.16±37.64 [a,b]	0.48±0.60 [a,b]	1.22±1.13 [a,b]
14 μM L	34.54±35.15 [a,b]	0.46±0.56 [a,b]	1.17±1.05 [a,b]
0.5 μM L+H_2O_2	44.72±39.91 [a,b]	0.66±0.65 [a,b]	1.59±1.14 [a,b]
1 μM L+H_2O_2	44.14±37.47 [a,b]	0.63±0.60 [a,b]	1.43±1.09 [a,b]
2 μM L+H_2O_2	34.74±32.54 [a,b]	0.42±0.48 [a,b]	1.18±0.92 [a,b]
4 μM L+H_2O_2	37.75±35.35 [a,b]	0.48±0.51 [a,b]	1.30±0.97 [a,b]
8 μM L+H_2O_2	46.65±36.66 [a,b]	0.57±0.52 [a,b]	1.27±0.90 [a,b]
10 μM L+H_2O_2	47.39±38.38 [a,b]	0.63±0.56 [a,b]	1.44±1.00 [a,b]
12 μM L+H_2O_2	35.61±35.87 [a,b]	0.45±0.54 [a,b]	1.09±1.01 [a,b]
14 μM L+H_2O_2	34.45±29.10 [a,b]	0.54±0.62 [a,b]	2.08±1.24 [a,b]

* DNA damages were expressed as tail intensity, tail moment, and tail migration in the lymphocytes of three separate studies. Results were given as the mean ± standard deviation. [a]$p<0.05$, compared with the negative control (1% DMSO); [b]$p<0.05$ compared with the positive control (0.1 mM H2O2).

were found to be higher than the H_2O_2-treated lymphocytes. This result has indicated that DNA damage is the result of the increases in the levels of Fpg sensitive sites showing the oxidized purine base damage in the lymphocytes.

TABLE III
The effects of Fpg and lycopene (L) on H_2O_2-induced oxidative DNA damage in human lymphocytes*

	Tail intensity	**Tail moment**	**Tail migration**
(-) Control	3.06±4.99	0.02±0.03	0.04±0.07
(-) Control+Fpg(+)	2.00±4.34	0.01±0.03	0.03±0.07
(+) Control (H_2O_2)	41.55±29.93 [a]	0.53±0.51 [a]	1.85±0.84 [a]
(+) Control+Fpg(+)	64.62±33.92 [c,e]	1.35±0.85 [c,e]	3.18±1.35 [c,e]
0.5 μM L	3.85±6.57 [b]	0.02±0.05 [b]	0.05±0.09 [b]
0.5 μM L+Fpg(+)	1.92±3.86 [d]	0.01±0.03 [d]	0.05±0.09 [d]
2 μM L	4.26±6.96 [b]	0.03±0.06 [b]	0.09±0.24 [b]
2 μM L+Fpg(+)	4.82±7.68 [d]	0.04±0.08 [d]	0.14±0.39 [d]
4 μM L	2.66±5.77 [b]	0.02±0.06 [b]	0.09±0.23 [b]
4 μM L+Fpg(+)	3.86±6.37 [d]	0.02±0.04 [d]	0.09±0.19 [d]
8 μM L	6.41±10.19 [b]	0.05±0.10 [b]	0.20±0.41 [b]
8 μM L+Fpg(+)	5.87±9.18 [d]	0.04±0.08 [d]	0.19±0.37 [d]
10 μM L	4.59±9.74 [b]	0.04±0.12 [b]	0.18±0.37 [b]
10 μM L+Fpg(+)	9.17±18.11 [c,d,e]	0.10±0.26 [d,e]	0.33±0.60 [c,d,e]
12 μM L	4.70±9.07 [b]	0.03±0.06 [b]	0.14±0.24 [b]
12 μM L+Fpg(+)	12.23±21.20 [c,d,e]	0.14±0.29 [c,d,e]	0.40±0.59 [c,d,e]
0.5 μM L+H_2O_2	30.31±23.10 [a,b]	0.42±0.39 [a,b]	1.67±0.81 [a,b]
0.5 μM L+H_2O_2+Fpg(+)	38.12±30.41 [c,d,e]	0.57±0.55 [c,d,e]	1.86±1.23 [c,d,e]
2 μM L+H_2O_2	34.97±25.08 [a,b]	0.50±0.45 [a,b]	1.93±0.82 [a,b]
2 μM L+H_2O_2+Fpg(+)	40.67±31.37 [c,d,e]	0.63±0.58 [c,d,e]	2.17±1.24 [c,d,e]
4 μM L+H_2O_2	35.34±29.07 [a,b]	0.45±0.48 [a,b]	1.77±0.80 [a,b]
4 μM L+H_2O_2+Fpg(+)	41.73±32.49 [c,d,e]	0.70±0.66 [c,d,e]	2.13±1.31 [c,d,e]
8 μM L+H_2O_2	38.65±22.96 [a,b]	0.57±0.40 [a,b]	2.09±0.60 [a,b]
8 μM L+H_2O_2+Fpg(+)	35.14±28.71 [c,d]	0.61±0.61 [c,d]	2.17±1.28 [c,d]
10 μM L+H_2O_2	34.37±28.83 [a,b]	0.45±0.46 [a,b]	1.76±0.82 [a,b]
10 μM L+H_2O_2+Fpg(+)	48.70±32.85 [c,d,e]	0.78±0.63 [c,d,e]	2.27±1.12 [c,d,e]
12 μM L+H_2O_2	37.54±25.47 [a,b]	0.51±0.44 [a,b]	1.98±0.67 [a,b]
12 μM L+H_2O_2+Fpg(+)	44.85±34.26 [c,d,e]	0.68±0.60 [c,d,e]	1.94±1.14 [c,d]

* DNA damages were expressed as tail intensity, tail moment, and tail migration in the lymphocytes of three separate studies. Results were given as the mean ± standard deviation. [a]$p<0.05$, compared to negative control for the standard comet assay; [b]$p<0.05$, compared to positive control for the standard comet assay; [c]$p<0.05$, compared to negative control for the Fpg-modified comet assay; [d]$p<0.05$, compared to positive control for the Fpg-modified comet assay; [e]$p<0.05$, standard comet assay was compared to Fpg-modified comet assay.

At the concentrations above 10 μM of lycopene alone, the levels of Fpg sensitive sites in the lymphocytes seemed to be increased in dose dependent suggesting that the increase in DNA damage seen above 10 μM lycopene is the result of oxidized purine base damage (Table III).

It is well known that free radical damage is closely connected with the development of a wide range of degenerative diseases such as atherosclerosis, cancer, and aging. Phenolic substances in natural products play an important role in protecting the organism against harmful effects of reactive oxygen species. In the last decade, the use of phenolic phytochemicals and herbal products for antioxidant purposes has been increased and a vast number of products have entered in the market all over the world. However, the toxicity, mutagenicity, and/or carcinogenicity of these phenolic compounds should be taken account of. There is no sufficient information about the potential risk of herbal products on human health. There are some conflicting studies that in *in-vitro* test systems, some herbal compounds are mutagenic and induce DNA damage.

Dietary intakes of tomatoes and tomato products containing lycopene have been shown to be associated with the decreased risk of chronic diseases such as cancer and cardiovascular diseases in numerous studies[14-17]. Serum and tissue lycopene levels have also been inversely related to the risk of lung and prostate cancers. Lycopene functions as a very potent antioxidant, and this is clearly a major important mechanism of lycopene action. Lycopene can trap singlet oxygen and reduce mutagenesis. However, evidence is accumulating for other mechanisms as well. Lycopene at physiological concentrations can inhibit human cancer cell growth by interfering with growth factor receptor signaling and cell cycle progression specifically in prostate cancer cells without evidence of toxic effects or apoptosis of cells[18,19].

The intake of some fruits or vegetables has been shown to cause significant reduction in oxidative damage to DNA. Lycopene was found to prevent oxidative damage to DNA and cell membranes and markedly reduced the histological changes induced by free radicals in rat liver.[20,21] Lycopene was reported to have an effect on the prevention of oxidative damage in many cells including lymphocyte DNA[22,23]. The supplementation of the diet with tomato, carrot or spinach products was reported to result in significantly decreased levels of endogenous strand breaks in the lymphocyte DNA[24].

It has been suggested that heat treated and processed tomato having high lycopene levels is related to the increases in the antioxidant and antiinflammatuar effects, therefore it ameliorate the carcinogenic detoxification via scavenging free radical[25].

In this study consistent with most of the studies, it was found that 0.5-20 mM lycopene had antioxidant activity in trolox method[27,28]. In the recent study of Chiste et al[26] demonstrated that at the lower concentration of lycopene prevented the oxidative damage in human erythrocytes. Lycopene (IC50=2.2±0.4μM) was found to inhibit the lipid peroxidation induced by tert-butyl hydroperoxide. However surprisingly lycopene did not inhibit hemoglobin oxidation or lipid peroxidation when induced by 2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH), even at the highest tested concentration.

In our study, the modulating effects of lycopene against the DNA damage induced by H_2O_2 in human lymphocytes were investigated by single cell gel electrophoresis. At the concentrations above 12 mM lycopene significantly induced DNA damage in human lymphocytes, but at the lower concentrations it did not induce DNA strand breakage. Even above the concentration of 12 μM that caused DNA damage alone, lycopene seemed to have a protective effect against H_2O_2 induced DNA breakage. At lower concentrations of lycopene (1-3 mM) seemed to have protective effect on DNA damage induced by xanthine-7-oxidase in HT29 cells. However at higher concentrations (4-10 mM), lycopene did not have protective effects on oxidative damage[29].

In our previous study, we found that a daily dose of 100 mg/kg lycopene for 14 days ameliorated the DNA damage and oxidative stress induced by an experimental obstructive jaundice in the peripheral lymphocytes, the whole blood cells, and the liver and kidney tissues of rats[30].

In a study on the healthy individuals, it has been demonstrated that the DNA damage in the lymphocytes significantly decreased after 8 weeks 12 mg/day lycopene supplementation[31].

In another study on 10 healthy women, the total lycopene concentration increased 0.5 mmol/L in the group eating lycopene, while decreased 0.2 mmol/L in the group without lycopene feeding. DNA damage from the treatment of H_2O_2 decreased % 33 and 42 % after the diet for the group feeding with lycopene and without lycopene, respectively[32].

In our study, consistent with other few *in vitro* studies, it seemed that lycopene had antioxidant activities at the lower concentrations, but had oxidant activities at the higher concentrations.

Conclusions

Modulation of carcinogenic and mutagenic effects by promoters or inhibitors from plant origin has been crucial importance for the final outcome of some biological effects, particularly for cancer since available epidemiological evidence is exceptionally strong and consistent between consumption of high amounts of vegetables and fruits and a reduced risk of cancer.

In our study we have shown that lycopene exerts both oxidant and antioxidant properties in human lymphocytes in vitro. Lycopene might have protective effects on H_2O_2-induced oxidative DNA strand breakage. In appropriate levels as a dietary supplement, lycopene can be used as a natural antioxidant. Lycopene seems to have therapeutic potential as interventions for numerous clinical disorders. In conclusion, it seems that our results highlight the potential benefit lycopene as a dietary supplement and as a natural antioxidant. However additional animal and human studies should be performed in order to clarify the mutagenic/antimutagenic potential of lycopene.

Acknowledgements

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Summary

Lycopene, a carotenoid compound widely present in tomatoes, processed tomato products and other fruits, plays an important role in

human diet. It has been shown that lycopene could act as an antioxidant and protect cellular macromolecules against oxidative stress. Dietary intake of food containing lycopene has been estimated to contribute to the inhibition of the diseases believed to be initiated by reactive oxygen species including atherosclerosis, cataract, age-related macular degeneration, multiple sclerosis, and cancer. The present study was carried out to investigate the modulating effects of lycopene against the oxidative DNA damage induced by H_2O_2 and the DNA damage was evaluated by single cell gel electrophoresis (comet assay). Formamidopyrimidine-DNA-glycoslase (Fpg) enzyme was used to examine the levels of oxidized purine bases induced by H_2O_2 in human lymphocytes. The antioxidant capacity of lycopene was also analyzed by trolox equivalent antioxidant capacity (TEAC) assay. At all concentrations studied above 10 mM lycopene alone seemed to induce DNA damage. However at the concentrations of 0.5-12 mM lycopene significantly reduced DNA single strand breakages induced by 0.05 mM H_2O_2 in human lymphocytes. At the concentrations above 10mM lycopene alone seemed to induce Fpg sensitive sites indicating the increased oxidized purine base levels. In conclusion, it seems that lycopene exerts both oxidant and antioxidant properties in human lymphocytes *in vitro*. In appropriate levels as a dietary supplement, lycopene can be used as a natural antioxidant but our results concern only *in vitro* experiments with human lymphocytes. Additional animal and human studies should be performed in order to clarify the mutagenic/antimutagenic potential of lycopene.

Key Words: Lycopene, hydrogen peroxide, single cell gel electrophoresis, comet assay, TEAC (trolox equivalent antioxidant capacity) assay

Özet

İnsan Lenfositlerinde Likopenin Oksidatif DNA Hasarı Üzerine Etkisi

Likopen domates, işlenmiş domates ürünleri ve diğer meyvelerde yaygın olarak bulunan ve insan diyetinde önemli rol oynayan bir karotenoiddir. Bir antioksidan olarak rol oynayabildiği ve oksidatif strese karşı hücresel makromolekülleri koruyabildiği gösterilmektedir. Likopen içeren gıdaların diyetsel alımlarının reaktif oksijen radikalleri tarafından

başlatıldığı düşünülen ateroskleroz, katarakt, yaşlanma ile ilişkili makula dejenerasyonu, multiple skleroz ve kanser gibi hastalıkların inhibisyonuna katkı sağladığı tahmin edilmektedir. Bu çalışma H_2O_2 ile indüklenen oksidatif DNA hasarına karşı likopenin etkisini incelemek üzere gerçekleştirilmiştir ve DNA hasarı tek hücre jel elektroforez (comet yöntemi) ile değerlendirilmiştir. İnsan lenfositlerinde H_2O_2 ile indüklenen okside pürin baz düzeylerini incelemek üzere formamidoprimidin-DNA-glikozilaz (Fpg) enzimi kullanılmıştır.

Troloks eşdeğer antioksidan kapasite (TEAK) yöntemi ile likopenin antioksidan kapasitesi belirlendi. 10 mM üzeri çalışılan tüm konsantrasyonlarda likopenin tek başına DNA hasarını indüklediği görülmüştür. Ancak insan lenfositlerinde 0.05 mM H_2O_2 ile indüklenen DNA tek zincir kırıklarını 0.5-12 mM konsantrasyonlarda likopen anlamlı olarak azaltmıştır. 10mM üzeri konsantrasyonlarda tek başına likopenin artmış okside pürin düzeylerin göstergesi olan Fpg duyarlı bölgeleri indüklediği görülmektedir. Sonuç olarak, likopen *in vitro* insan lenfositlerinde hem antioksidan hem de oksidan özellik göstermektedir. Diyetsel destek olarak uygun düzeylerde likopen doğal bir antioksidan olarak kullanılabilir, fakat sonuçlarımız sadece insane lenfositleri ile yapılmış *in vitro* deneylerle alakalıdır. Likopenin mutajenik/antimutajenik potansiyelini aydınlatmak için ayrıca hayvan ve insan çalışmaları da gerçekleştirilmelidir.

Anahtar Kelimeler: Likopen, hidrojen peroksit, tek hücre jel elektroforez, comet yöntemi, TEAK (Troloks eşdeğer antioksidan kapasite) yöntemi

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