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Antioxidant and Hepatoprotective Effects of Tomato Extracts

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Abstract - The objective of present study was to investigate the antioxidative and hepatoprotective effects of tomato extracts. Total antioxidant capacity and total antioxidant response were 5.5 and 19.8μg Trolox equivalent per mg of tomato extract, respectively. DPPH radical scavenging activity of tomato extracts (10mg ml^{-1}) was 70% as compared to 100% by pyrogallol solution as a reference. The effect of the tomato extracts on lipid peroxidation was examined using rat liver mitochondria induced by iron/ascorbate. Tomato extracts at the concentration of 0.5mg ml^{-1} significantly decreased TBARS concentration. Tomato extracts prevented lipid peroxidation in a dose-dependent manner. The effect of the tomato extracts on reactive oxygen species (ROS) generation was examined using cell-free system induced by $H_2O_2/FeSO_4$. Addition of 1mg ml^{-1} of tomato extracts significantly reduced dichlorofluorescein (DCF) fluorescence. Tomato extracts caused concentration-dependent attenuation of the increase in DCF fluorescence, indicating that tomato extracts significantly prevented ROS generation *in vitro*. The effect of tomato extracts on cell viability and proliferation was examined using hepatocyte culture. Primary cultures of rat hepatocytes were incubated with 1mM tert-butyl hydroperoxide (t-BHP) for 90 min in the presence or absence of tomato extracts. MTT values by addition of tomato extracts at the concentration of 2, 10, and 20mg ml^{-1} in the presence of t-BHP were 13, 33 and 48%, respectively, compared to 100% as control. Tomato extracts increased cell viability in a dose-dependent manner. These results demonstrate that tomato extracts suppressed lipid peroxidation and t-BHP-induced hepatotoxicity and scavenged ROS generation. Thus antioxidant and hepatoprotective effects of tomato extracts seem to be due to, at least in part, the prevention from free radicals-induced oxidation, followed by inhibition of lipid peroxidation.

Key words - Tomato extracts, Lipid peroxidation, Cytotoxicity, ROS, Rat hepatocytes

Introduction

Antioxidants play important role in reducing the risk of free radical-related oxidative damage associated with degenerative diseases. The role of dietary antioxidants such as carotenoid is of significant interest. A number of epidemiological studies have suggested that an increased dietary intake of carotenoid-rich fruits and vegetables is associated with a reduced incidence of certain cancers (Block *et al.*, 1992; Van Poppel and Goldbohm, 1995). Carotenoids are pigment widely distributed in animals and plants. The major dietary carotenoids found in plasma and tissues are α-caroteine, β-caroteine, β-cryptoxanthin, lutein and lycopene. Lycopene is the major carotenoid in the tomato. Inverse relationship between tomato consumption and prostate cancer has been reported (Giovannuci *et al.*, 1995). This anti-cancer effect appears to be attributed to ability of lycopene found in the tomato to scavenge free radicals (Di Mascio *et al.*, 1989; Sies and Stahl, 1998).

It is well known that reactive oxygen species (ROS) production from mitochondria by oxidative stress can cause damage to lipids, proteins and DNA. Oxidative stress has been implicated in numerous diseases, including atherosclerosis, cancer, neurodegenerative diseases and aging (Thannickal and Fanburg, 2000). The biological activity of tomato products or lycopene in disease prevention was postulated on the ability of lycopene to protect against oxidative damage (Rao and Agarwal, 1998; Sies and Stahl, 1998). It is reported that lycopene also increased activities of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase or glutathione reductase. However lycopene afforded significant protection against oxidative stress and antioxidant enzyme activities at lower concentration but at higher concentrations these protection and activities rapidly declined (Breinholt *et al.*, 2000).

Anticarcinogenic potential and protection against DNA damage of lycopene or tomato extracts have been extensively studied. However hepatoprotective and antioxidant properties of tomato extracts have not been studied well. The objective of this study was to examine the protective effect of tomato extracts on the tert-butyl hydroperoxide (t-BHP)-induced cytotoxicity in rat hepatocytes. In addition, the antioxidant effects of tomato extracts on ROS production, free radical scavenging and lipid peroxidation were evaluated.

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Materials and Methods

Chemicals

2',7'-Dichlorofluorescin-diacetate (DCFH-DA) was purchased from Molecular Probes, Inc. (Eugene, OR, USA). Williams's medium E and Hanks' balanced salt solution were purchased from Invitrogen Corp. (Gaithersburg, MD, USA). Fetal bovine serum was purchased from Combrex Bio Science Inc. (Walkersville, MD, USA). Six-well tissue culture plates were purchased from Becton Dickinson and Company (Franklin Lakes, NJ, USA). All of the other chemicals and solvents used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

Male Sprague Dawley rats, approximately 180~200g BW from Daehan Biolink Co. Ltd (Eumsung, Chungbuk, Korea) were acclimatized for 1 week with ad libitum access to standard rodent chow and tap water before liver perfusion or mitochondria isolation.

Extraction of tomato

The regular red tomatoes were purchased from local market. Tomatoes were sliced and blended in food processor for 5 min to obtain tomato slurry. One hundred fifty grams of tomato slurry were extracted with 80% acetone (1:2 w/v), filtered and then evaporated. The tomato extracts was kept at -20℃ until analysis.

Total antioxidant capacity (TAC) measurement

Total antioxidant status of the sample was determined using TAC method (Erel, 2004a) in which a colorless molecules, reduced 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) is oxidized to a green $ABTS^{\cdot+}$ using hydrogen peroxide in acidic buffer solution and antioxidants present in the sample accelerated the bleaching rate to a degree proportional to their concentrations. This reaction was monitored by spectrophotometry at 666nm. Trolox was used as a standard for TAC measurement and the assay results were expressed as in terms of μg Trolox equivalent mg^{-1} sample.

Total antioxidant response (TAR) measurement

TAR of tomato extracts against potent free radical reactions was measured according to the method of Erel (2004b). Potent free radical reactions were initiated with the production of hydroxyl radical ($OH^{\cdot}$) via Fenton reaction. Antioxidants in the sample prevent the reaction by potent free radicals which oxidize to colorless o-dianisidine molecules to yellow-brown colored dianisidy radicals at low pH. This reaction was monitored by spectrophotometry at 444nm. The assay results were expressed in μg Trolox equivalent mg^{-1} sample.

Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The DPPH radical scavenging activity was measured using a modification of the method of Malterud *et al.* (1993). DPPH (45μg ml^{-1}) was rapidly mixed with tomato extracts. The decline in radical concentration was monitored as the decrease in absorbance at 515nm over a period of 5 min. Pyrogallol solution (125μg ml^{-1}) was used as a reference corresponding to 100% radical scavenging activity.

Mitochondrial isolation

Rat liver mitochondria were isolated from male Sprague-Dawley rats by the method of Hovius *et al.* (1990) in a medium containing 250mM sucrose, 5mM HEPES and 0.1% BSA, adjusted to pH 7.4.

Lipid peroxidation

The influence of tomato extracts on mitochondrial lipid peroxidation induced by iron-ascorbate was determined by the method of Sanz *et al.*(1994) with some modifications. Rat liver mitochondria (0.5mg ml^{-1}) were incubated with 10uM $FeSO_4$ and 100μM ascorbate in 50mM Tris, pH 7.4 in the absence or presence of tomato extracts at 37℃ for 60 min. After the incubation, lipid peroxidation was measured by the formation of thiobarbituric acid reactive substances (TBARS).

TBARS determination

The extent of lipid peroxidation was determined by measuring the release of TBARS. TBARS were determined according to the procedure of Stacey and Klaassen (1981) with slight modifications. Mitochondrial suspension was centrifuged at 12,000 rpm for 5 sec. Aliquots of the supernatant were added to a tube containing 2.8% trichloroacetic acid, 0.05% butylated hydroxytoluene and 0.37% thibarbituric acid. The mixture was heated at 90℃ for 20 min. After cooling, the mixture was centrifuged and supernatant was read on fluorescence plate reader with an excitation wavelength at 530nm and an emission wavelength at 590nm. Standards were prepared from 1,1,3,3-tetraethoxypropane, yielding a quantitative adduct of the malonaldehyde-TBARS product.

ROS generation using cell-free system

2',7'-dichlorofluorescin (DCFH) was prepared from DCFH-DA (Cathcart *et al.*, 1983). ROS were generated by 1μM H_2O_2 and 10μM $FeSO_4$. Tomato extracts was added to the reaction mixture. DCFH ox-

idation by ROS to dichlorofluorescein (DCF) was quantified from the fluorescence emission intensity with an excitation wavelength at 488nm and an emission wavelength at 525nm at 37℃. A decrease in fluorescence indicates oxygen free radical scavenging activity of the tomato extracts.

Hepatocyte Culture

Hepatocytes were isolated from adult male Sprague-Dawley rats by the two-step collagenase perfusion method of Seglen (1976). Hepatocytes were plated at a density of 5×10^5 cells ml^{-1} into 6-well tissue culture plates in William's E medium with 10% fetal bovine serum, 1mM dexamethason, 1mM insulin, 200mM L-glutamin, 100 U ml^{-1} penicillin and 0.1mg ml^{-1} streptomycin. The cultures were incubated at 37℃ in 5% CO_2 in air for 5 hr. The cells were washed with fresh serum-free William's E medium and then subjected to treatments (control, 1mM t-BHP or 1mM t-BHP with tomato extracts) at 37℃ for 90 min in CO_2 incubator.

Cytotoxicity determination

Cytotoxcity or cell viability was determined using a modification of the 3-(4,5-dimethylthiazol-2-ul)-2,5-diphenyl tetrazolium bromide (MTT) method (Mosmann, 1983). Following exposure of hepatocytes to t-BHP in the absence or presence of the tomato extracts, monolayer cells were washed with cold PBS. One hundred ul of MTT (5mg ml^{-1}) were added, and samples were incubated for 3 hr at 37℃. The cells were lysed and solubilized by addition of 1ml of 0.04M HCl and the absorbance of aliquouts was determined at 570nm. Cell viability (%) was calculated relative to control.

Protein concentration determination

Protein concentration was measured using the method of Bradford (1976). Bovine serum albumin was used as the standard.

Statistical analysis

Data were subjected to analysis of variance. The mean values among treatment groups were compared by Duncan's multiple range test (Steel and Torrie, 1980). Statistical differences were considered to be significant at $p<0.05$.

Results

After extraction of 150 g of fresh tomato 4.77g of acetone tomato extract was obtained. The results of total antioxidant status of the tomato extracts were shown in Table 1. TAC activity of the tomato extracts was 5.5μg Trolox equivalent on the basis of the dry weight (mg) of tomato extract. TAR activity of the tomato extracts was 19.8μg Trolox equivalent per mg of tomato extract. DPPH radical scavenging activity of tomato extracts (10mg ml^{-1}) was 70% as compared to 100% by pyrogallol solution as a reference.

Table 1. Antioxidant status of the tomato extracts*

TAC (μg Trolox equiv. mg^{-1})	TAR (μg Trolox equiv. mg^{-1})	DPPH (%)
5.5 ± 0.49	19.8 ± 1.31	70 ± 1.7

*Values are presented as the means±SE derived from four determinations.

The effect of the tomato extracts on lipid peroxidation in rat liver mitochondria induced by iron/ascorbate is shown in Fig. 1. Lipid peroxidation was examined by measuring TBARS concentration. As expected, treatment of liver mitochondria with iron/ascorbate caused to increase TBARS concentration. Tomato extracts at the concentration of 0.5mg ml^{-1} significantly decreased TBARS concentration. TBARS concentration continued to decrease as the concentration of tomato extracts increased, indicating that tomato extracts prevented lipid peroxidation in a dose-dependent manner. No further inhibition of lipid peroxidation was observed by tomato extracts at the concentration more than 2mg ml^{-1}.

The effect of the tomato extracts on ROS generation induced by $H_2O_2/FeSO_4$ in cell-free system is shown in Fig. 2. $H_2O_2/FeSO_4$ caused to increase DCF fluorescence. Addition of 1mg ml^{-1} of tomato extracts significantly reduced DCF fluorescence. DCF fluorescence

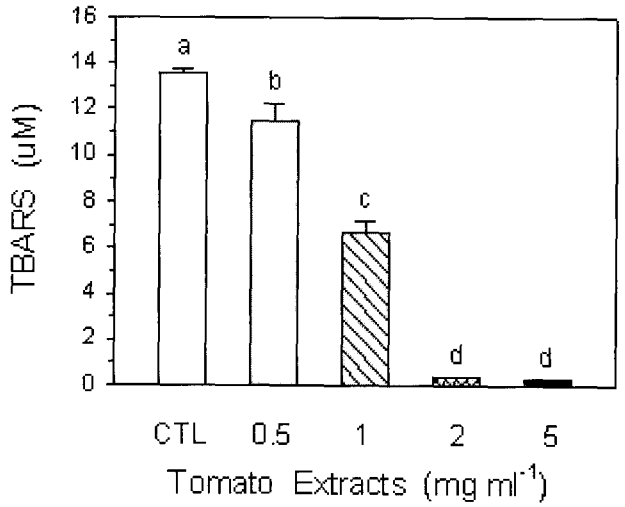

Fig. 1. The effect of the tomato extracts on lipid peroxidation in rat liver mitochondria. Rat liver mitochondria were incubated with iron/ascorbic acid in the absence (control) or presence of various concentrations of tomato extracts. Lipid peroxidation was determined by measuring the release of TBARS. Each bar represents the mean±SE derived from four determinations. [a,b,c,d]Values with different letters are significantly different ($p<0.05$).

was further decreased as tomato extracts added increased, resulting in a concentration-dependent attenuation of the increase in DCF fluorescence. Tomato extracts at the concentration of 10mg ml^{-1} suppressed DCF fluorescence by 62%, demonstrating that tomato extracts significantly prevented ROS generation *in vitro*.

The effect of tomato extracts on viability and proliferation of hepatocytes was examined using MTT assay, as shown in Fig 3. Cell viability was 7.5% by exposure of hepatocyte to 1mM of t-BHP for 90 min, indicating that cytoxicity was induced by the t-BHP. Cell viability by addition of tomato extracts at the concentration of 2 and 10mg ml^{-1} in the presence of t-BHP were 13% and 33%, respectively. Cell viability continued to increase as the concentration of tomato extracts increased, indicating that tomato extracts increased Cell viability in a dose-dependent manner. However cell viability by addition of 20mg ml^{-1} of tomato extracts was 48% compared to control, indicating that tomato extracts significantly increased but not fully recovered cel viability against cytotoxicity induced by t-BHP in the present study.

Discussion

The results show that tomato extract protects hepatotoxicity against t-BHP and scavenges ROS generation. These data also confirmed the previous finding that tomato products or lycopene protected lipid peroxidation against oxidative stress.

It has been reported that the consumption of tomato products improved antioxidant capacity. The antioxidant effect of tomato products is ascribed to lycopene, the predominant carotenoid. TAC and TAR activities of the tomato extracts were 5.5 and 19.8μg Trolox equivalent on the basis of mg of tomato extract, respectively (Table 1). These strong antioxidant status of tomato extract was also observed in DPPH radical scavenging activity at the concentration of 10mg ml^{-1}, which was 70% as compared to 100% by pyrogallol solution as a reference.

Several studies have demonstrated that lycopene prevented lipid peroxidation (Leal *et al*, 1999; Matos *et al*., 2000, 2001) and oxidative damage to DNA in liver (Matos *et al*., 2000, 2001). The protection against lipid peroxidation was confirmed in the present study, in which tomato extracts as low as 0.5mg ml^{-1} of concentration significantly decreased TBARS concentration of rat liver mitochondria induced by iron/ascorbate and further prevented lipid peroxidation in a dose-dependent manner (Fig. 1).

Oxidative stress has been considered to be responsible for various chronic diseases including atherosclerosis, cancer, neurodegenerative diseases and aging. ROS production from mitochondria by oxidative stress can cause damage to lipids, proteins and DNA. Lycopene had the capacity to quench singlet oxygen (Di Mascio *et al*., 1989) and scavenge peroxyl radicals (Sies and Stahl, 1998). In this study using cell-free system tomato extracts significantly attenuated DCF fluo-

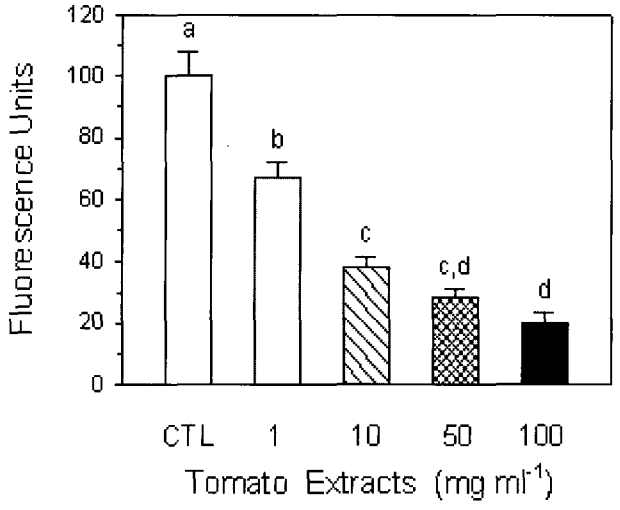

Fig. 2. The effect of the tomato extracts on ROS generation. DCFH oxidation to DCF by iron/hydrogen peroxide-induced ROS generation in the absence (control) or presence of various concentrations of tomato extracts was measured. Each bar represents the mean±SE derived from four determinations. [a,b,c,d]Values with different letters are significantly different ($p<0.05$).

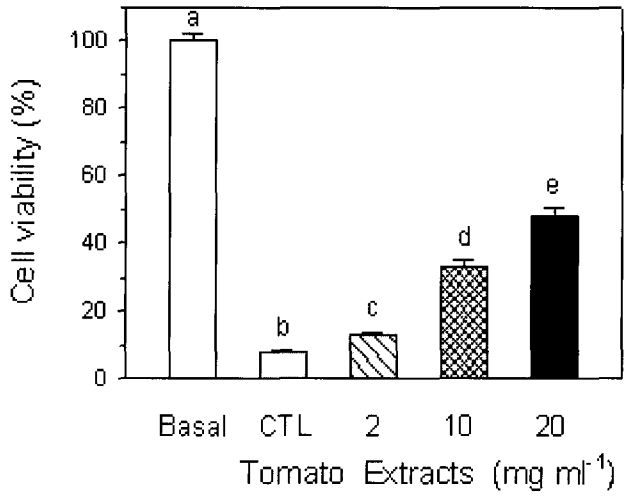

Fig. 3. The effect of the tomato extracts on hepatocyte viability. Hepatocytes were cultured for 90 min in the presence of 1mM t-BHP and various concentrations of tomato extracts. In basal group hepatocytes were maintained in the absence of t-BHP and tomato extracts. Cell viability was determined using MTT method. Each bar represents the mean±SE derived from four determinations. [a,b,c,d,e]Values with different letters are significantly different ($p<0.05$).

rescence induced by $H_2O_2/FeSO_4$, demonstrating that tomato extracts significantly prevented ROS generation *in vitro* (Fig. 2). Recently Xu *et al.* (2003) have reported that lycopene significantly decreased ethanol-induced hydrogen peroxide production in HepG2 cells expressing CYP2E1. Leal *et al.* (1998) have demonstrated that lycopene reduced the cytotoxicity of T-2 mycotoxin in hepatocytes. In this study t-BHP was applied to hepatocytes to induce cytotoxicity. Tomato extracts significantly increased cell viability against cytotoxicity induced by t-BHP (Fig. 3).

In summary, this study demonstrates that tomato extracts suppress lipid peroxidation and t-BHP-induced hepatotoxicity as well as scavenge ROS generation. Thus antioxidant and hepatoprotective effects of tomato extracts seem to be due to, at least in part, the prevention from free radicals-induced oxidation, followed by inhibition of lipid peroxidation. These findings may contribute toward a better understanding of biochemical mechanisms associated with lycopene protection against oxidative stress.

Acknowledgements

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