Free Radical Research, April 2005; 39(4): 449–455

Lycopene, tomatoes, and coronary heart disease

SAMARJIT DAS, HAJIME OTANI, NILANJANA MAULIK, & DIPAK K. DAS

Cardiovascular Research Center, University of Connecticut School of Medicine, Farmington, CT, USA

Accepted by Professor E. Niki

(Received 16 October 2004; in revised form 17 December 2004)

Abstract
Tomato and its major antioxidant component lycopene have recently been focused as important antioxidant nutrients because of their ability to reduce reactive oxygen species and to provide health benefits. Most of the studies were undertaken to determine the usefulness of lycopene against cancer and cardiovascular diseases. Epidemiological studies, however, yielded conflicting results. This study was undertaken to compare cardioprotective abilities of tomato juice and lycopene. Rats were gavaged either tomato juice or lycopene for 3 weeks. At the end of 3 weeks, isolated hearts were subjected to 30 min ischemia followed by 2 h of reperfusion. Both tomato juice and lycopene reduced the extent of lipid peroxidation; but only tomato juice, but not lycopene, improved post-ischemic ventricular function, and reduced myocardial infarct size and cardiomyocyte apoptosis. The results indicated for the first time that tomato juice, but not lycopene, possesses cardioprotective ability.

Keywords: *Lycopene, tomato juice, antioxidant, reactive oxygen species, ischemia/reperfusion, apoptosis*

Introduction

Nutrition plays a crucial role in the development of many degenerative diseases including coronary heart disease (CHD), which is the major cause of deaths in the Western World. CHD is also a major contributor of morbidity and mortality in the developing countries. Although there are several risk factors for CHD, lifestyle factors like diet is considered as a major risk factor associated with cardiac abnormality [1]. Among many types of nutrition, antioxidant nutrients are recognized as major players for the prevention of CHD [2].

Among the antioxidant-rich foods, grapes occupy the top position because of its relation with red wine and French Paradox [3]. Grapes are rich in polyphenolic antioxidants, and contain two important cardioprotective antioxidants, proanthocyanidins and resveratrol [4,5]. Recently, lycopene has been focused as an antioxidant supplement because of its function as a free radical scavenger and its ability to maintain other antioxidant substances in the reduced form [6]. While the major source of lycopene is tomato, this vegetable contains many other antioxidants in addition to lycopene such as—carotene and lutein [7]. Since all of these antioxidants are likely to be beneficial for the heart, we sought to compare the cardioprotective abilities of lycopene and tomato. The results of our study demonstrated that both lycopene and tomato could reduce the extent of myocardial ischemic reperfusion injury, but tomato had better cardioprotective role than lycopene.

Materials and methods

Lycopene, from tomato, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lycopene, a red color crystalline solid, was dissolved in ethyl alcohol under boiling condition. Tomato juice was purchased from Campbell Soup Company (Camden, NJ, USA)

Correspondence: D. K. Das, Cardiovascular Research Center, University of Connecticut, School of Medicine, Farmington, CT 06030-1110, USA. Tel: 1 860 679 3687. Fax: 1 860 679 4606. E-mail: ddas@neuron.uchc.edu

ISSN 1071-5762 print/ISSN 1029-2470 online © 2005 Taylor & Francis Group Ltd
DOI: 10.1080/10715760500053685

under the brand name Campbell Original Tomato Juice.

Animals

Sprague Dawley rats of 250 gm body weight were used in this study. All animals used in this study received humane care in compliance with the principles of the laboratory animal care formulated by the National Society for Medical Research and Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication Number NIH 85-23, revised 1985). Sprague Dawley male rats weighing between 250–300 gm were fed *ad libitum* regular rat chow with free access to water until the start of the experimental procedure. The rats were randomly assigned to one of the following three groups: (i) Group I served as control was given water only; (ii) Group II was given tomato juice (1 ml juice/day), while (iii) Group III was fed lycopene (0.088 mg/day). Juice or water was fed by gavaging for 21 days. At the end of 3 weeks, the rats were sacrificed for the isolated heart preparation.

Isolated perfused heart preparation

Sprague Dawley rats weighing 275–300 g were anesthetized with pentobarbital (80 mg/kg, i.p. injection, Abbott Laboratories, North Chicago, IL). After intravenous administration of heparin (500 IU/kg, Elkins-Sinn Inc., cherry hill, NJ), the chests were opened, the hearts were rapidly excised and mounted on a non-recirculating Langendorff perfusion apparatus [8]. The perfusion buffer used in this study consisted of a modified Krebs-Henseleit bicarbonate buffer (KHB) (in mM: 118 NaCl, 4.7 KCL, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 10 Glucose and 1.7 $CaCl_2$), PH 7.4, gassed with 95% O_2–5% CO_2, and filtered through a 5 μm filter to remove any particulate contaminants. The buffer was maintained at a constant temperature of 37°C and was gassed continuously for the entire duration of the experiment. Left atrial cannulation was then carried out and after allowing for stabilization of 10 min in the retrograde perfusion mode, the circuit was switched to the antegrade working mode which allowed for the measurement of myocardial contractility as well as aortic and coronary flows, as described in detail in a previous paper [8]. Essentially it is a left heart preparation in which the heart perfused with a constant preload of 17 cm H_2O (being maintained by means of a Masterflex variable speed modular pump, Cole Parmer Instrument Company, Vernon Hills, IL) and pumps against an afterload of 100 cm H_2O. At the end of 10 min, after the attainment of steady state cardiac function, baseline functional parameters were recorded as usual. The circuit was then switched back to the retrograde mode. The isolated hearts were pre perfused with KHB buffer for 15 min, and then subjected to ischemia for 30 min followed by 2 h of reperfusion. Three Groups were studied: Group I: Ischemia/reperfusion-control, Group II: Ischemia/reperfusion-Tomato and Group III: Ischemia/reperfusion-lycopene. The first 10 min of reperfusion was in the retrograde mode to allow for post-ischemic stabilization and thereafter in the antegrade-working mode to allow for assessment of functional parameters, which were recorded at 10, 30, 60, 90 and 120 min into reperfusion.

Measurement of cardiac function

Aortic pressure was measured using a Gould P23XL pressure transducer (Gould Instrument System Inc., Valley View, OH) connected to sidearm of the aortic cannula [8]. The signal was amplified using a Gould 6600 series signal conditioner and monitored on CORDAT II real-time data acquisition and analysis system (Triton Technologies, San Diego, CA). Heart rate, developed pressure (defined as the difference of the maximum systolic and diastolic pressures) and the first derivative of developed pressure (dp/dt max) were all derived or calculated from the continuously obtained pressure signal. Aortic flow was measured using a calibrated flowmeter (Gilmont Instruments Inc., Barrington, IL) and coronary flow was measured by collection of the coronary effluent dripping from the heart.

Evaluation of infarct size

Hearts to be used for infarct size calculations were taken upon the end of experiment and 1% Triphenyl tetrazolium solution in phosphate buffer (Na_2HPO_4 88 mM, NaH_2PO_4 1.8 mM) was perfused into coronary artery directly for 15 min at 37°C and then stored at −70°C for subsequent processing. Frozen hearts (including only ventricular tissue) were sliced transversely in a plane perpendicular to the apical–basal axis into approximately 0.5 mm thick sections, blotted dry, placed in between microscope slides and scanned on a Hewlett-Packard Scanjet 5p single pass, flat bed scanner (Hewlett-Packard, Palo Alto, CA). Using the NIH 1.61 image processing software, each digitized was subjected to equivalent degrees of background subtraction, brightness and contrast enhancement for improved clarity and distinctness [9]. Risk (equivalent to total LV muscles mass) as well as infarct zone of each slice were traced and the respective areas were calculated in terms of pixels. The weight of each slice was then recorded to facilitate the expression of total and infarct masses of each slice in grams. The risk and infarct volumes in cm^3 of each slice were then calculated on the basis of slice weight to remove the introduction of any errors due to

non-uniformity of heart slice thickness. The risk volumes and infarct volumes of each slice were summed to obtain the risk and infarct volumes for the whole heart. Infarct size was taken to be the percent infarct volume/risk volume for any one heart.

Evaluation of apoptosis

Immunohistochemical detection of apoptosis cells were carried out using TUNEL in which residues of digoxigenin-labelled dUTP were catalytically incorporated into the DNA by terminal deoxynucleotidyl transferase II, an enzyme which catalyzes a template-independent addition of nucleotide triphosphate to the 3′OH ends of double- or single-stranded DNA [10]. The incorporated nucleotide was incubated with a sheep polyclonal anti-digoxigenin antibody followed by a FITC-conjugated rabbit anti-sheep IgG as a secondary antibody as described by the manufacturer (Apop Tag Plus, Oncor Inc., Gaithersburg. MD). The sections ($n = 5$) were washed in PBS three times, blocked with normal rabbit serum and incubated with mouse monoclonal antibody recognizing cardiac myosin heavy chain (Biogenesis Ltd., Poole, UK) followed by staining with TRIRC-conjugated rabbit anti-mouse IgG (200:1 dilution, Dako Japan, Tokyo, Japan). The fluorescence staining was viewed with a control and express as a percent of total myocyte population.

Measurement of ROS activities

Malonaldehyde (MDA) formation is a presumptive marker for lipid peroxidation indicative of the development of oxidative stress. MDA was estimated in the myocardium as described previously [9]. In short, the heart biopsy (0.2 mg) was homogenized in 2 ml of 20% trichloro acetic acid, 5.3 mM sodium bisulfite, and centrifuged at 7500*g* for 15 min. The supernatants were collected, derivatized with 2,4-dinitrophenylhydrazine (DNPH), and extracted with pentane. Aliquots of 25 μl in acetonitrile were injected onto a Beckman Ultrasphere C18 (3 mm) column. The products were eluted isocratically with a mobile phase containing acetonitrile–water–acetic acid (40:60:1, v/v/v) and measured at three different wave lengths (307, 325 and 356 nm) using a Waters M-490 multichannel UV detector. The peak for MDA was identified by co-chromatography with DNPH derivative of the authentic standard, peak addition, UV pattern of absorption at the three wavelengths, and by GC–MS.

Statistical analysis

To compare between three groups, ANOVA followed by Bonferroni test was used. To compare between two groups, Student's *t*-test was used. The results are expressed as Means ± SEM and considered statistically significant when $p < 0.05$.

Results

Body and heart weights by lycopene and tomato juice feeding

The effect of lycopene and tomato juice on the body and heart weights is shown in Table I. As shown in the Table I, lycopene had no effect on either body weight or heart weight after 3 weeks of feeding. In contrast, tomato juice significantly increased both body and heart weights, indicating that tomato juice feeding caused cardiac hypertrophy to some extent.

Functional recovery

There were no differences in baseline function between three groups. In general, there were no significant differences between tomato juice vs. control and also lycopene vs. control on heart rates and coronary flow (Table II). As was expected, upon reperfusion, the absolute values of all functional parameters were decreased in all the groups as compared with the respective baseline values. Group II (Tomato juice) displayed significant recovery of post ischemic myocardial function (Figure 1). The cardio protective effects of tomato juice were evidenced by significant differences in the LVDP from R-30 onwards (Table II), the difference is especially apparent at R-60 (110.98 + 4.48 mmHg vs. 88.01 + 9.57 mmHg) and at R-120 (83.22 + 6 mm Hg vs. 42.5 + 7.62 mm Hg) and also evidenced by the significant difference in the LVdp/dt at R-30 (2764.6 + 194.8 mm Hg/sec vs. 2365 + 34.74 mmHg/s) and onwards at R-60 (2219.3 + 229.44 mm Hg/s vs. 968.3 + 97.22 mm Hg/s) and also at R-120 (1412.17 + 133.6 mm Hg/s vs. 481 + 84.54 mm Hg/s). Aortic flow was markedly higher in the tomato juice group from R-30 onwards at the all rest three points at R-60 (32.88 + 8.25 ml/min vs. 9.19 + 2.39 ml/min) and at R-120 (10.38 + 2.6 ml/min vs. 2.5 + 0.41 ml/min). Lycopene fed rat hearts displayed recovery of post-ischemic myocardial function. These were evidenced by significantly higher

Table I. Effects of tomoto and lycopene on the body weight and heart weight

	Control	Tomato	Lycopene
Body weight (g)	317.5 ± 8.4	381.7 ± 10.5	315.5 ± 7.1
Heart weight (mg)	470 ± 13	680 ± 12.5	450 ± 8.5

Rats were fed [$n = 6$ per group] either tomato juice or lycopene by gavaging for 21 days. Control rats were given water only by gavaging. At the end of 21 days, the body weight was determined, the rats sacrificed, and the heart weights were recorded. The results are expressed as Means ± SEM of 6 rats per group.

Table II. Effects of tomato juice and lycopene on postischemic ventricular function

	Group	Baseline	10 min	30 min	60 min	120 min
Heart Rate (beats/min)	Control	328.65 ± 40.38	348.93 ± 13.1	309.567 ± 22.9	361.1 ± 27.4	414.28 ± 24.38
	Tomato	376.92 ± 28.02	325.832 ± 26	340.08 ± 16.37	347.23 ± 25.15	409.83 ± 12.54
	Lycopene	402.3 ± 19.67	374.575 ± 23.96	395.85 ± 20	419.73 ± 19.08	412.25 ± 31.19
LVDP (mm Hg)	Control	126.76 ± 3.16	107.43 ± 5.40	103.56 ± 7.10	88.01 ± 9.57	42.5 ± 7.62
	Tomato	125.35 ± 5.16	112.4 ± 4.24	116.47 ± 1.96	110.98 ± 4.48*	83.22 ± 6*
	Lycopene	133.83 ± 2.32	105.3 ± 6.32	116.475 ± 4.26	97.6 ± 4.2	67.55 ± 4.36
LVdp/dt (mmHg/s)	Control	3068.8 ± 132.02	2630.7 ± 121.9	2365 ± 34.74	968.3 ± 97.22	481 ± 84.54
	Tomato	3316.6 ± 216.56	2798.6 ± 194.8	2764.6 ± 170.94*	2219.3 ± 229.44*	1412.17 ± 133.6*
	Lycopene	3371 ± 78.59	2530.5 ± 207.89	2526.5 ± 323.5	1601 ± 191.68	862.25 ± 187.14*
Aotic flow (ml/min)	Control	71.55 ± 5.56	39.68 ± 6.21	35.31 ± 8.41	9.19 ± 2.39	2.5 ± 0.41
	Tomato	77.17 ± 8.2	57.6 ± 7.6	56.52 ± 7.5	32.88 ± 8.25*	10.38 ± 2.6*
	Lycopene	80.25 ± 1.88	30.53 ± 3	30.63 ± 4.65	13.14 ± 4.29	3.015 ± 0.76
Coronaryflow (ml/min)	Control	27.5 ± 1	24.9 ± 1.5	24.2 ± 2.4	22.1 ± 1.99	19.8 ± 1.87
	Tomato	24.1 ± 2.15	22.3 ± 2.12	21.9 ± 1.99	22.6 ± 2.32	21.2 ± 2.73
	Lycopene	24.9 ± 1.63	21.55 ± 1.67	23.1 ± 1.4	21.6 ± 2.12	18.75 ± 2

LVDP, left ventricular developed pressure; LVdp/dt, maximum first derivatives of developed pressure.
Results are expressed as mean ± SEM of 6 animals as group *$p < 0.05$ tomato or lycopene vs. control.

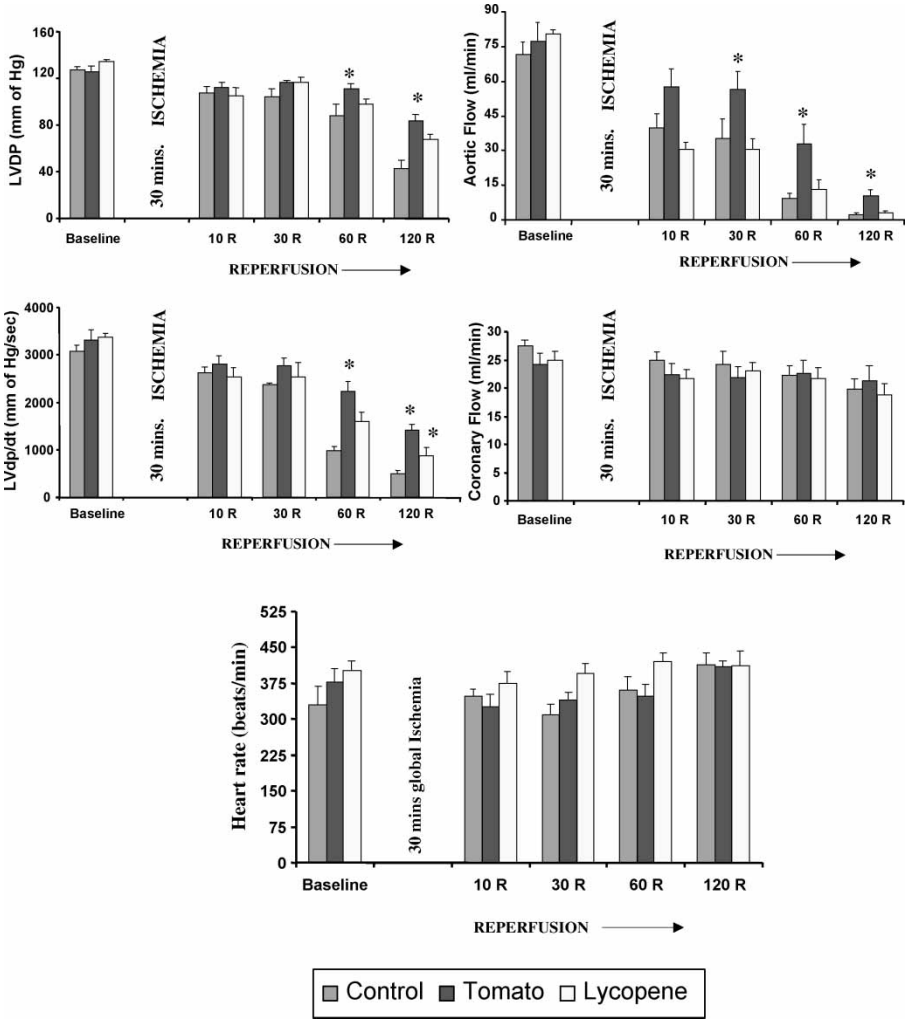

Figure 1. Effects of tomato juice and lycopene on the post-ischemic recovery of left ventricular function. Results are expressed as Means ± SEM of six hearts per group. *$p < 0.05$ vs. control or lycopene.

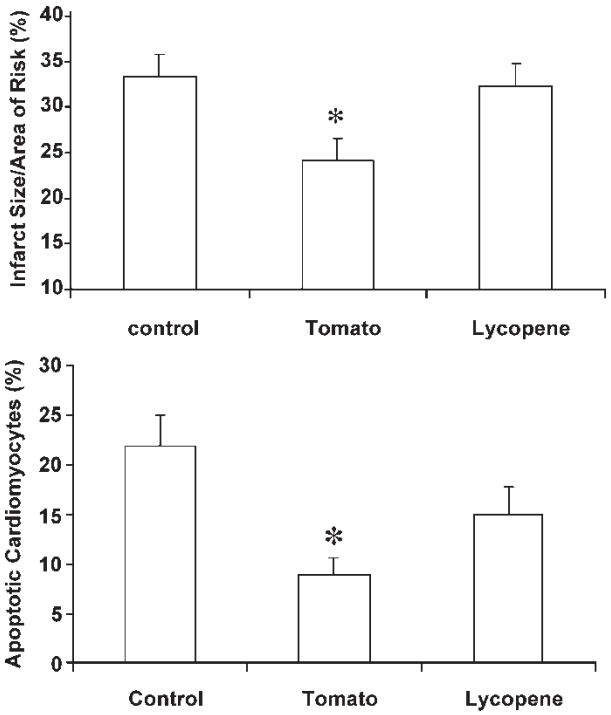

Figure 2. Effects of tomato juice and lycopene on myocardial infarct size and cardiomyocyte apoptosis in the hearts subjected to 30 min ischemia and 2 h of reperfusion. Results are expressed as Means ± SEM of six hearts per group. *$p < 0.05$ vs. control or lycopene.

LVdp/dt max readings at R-120 (862.25 + 187.14 mm Hg/s vs. 481 + 84.54 mm Hg/s). Left ventricular developed pressure and aortic flow were also higher than control values in lycopene treated group compared to control group. There were no differences between the groups for the heart rate.

Infarct size

In this study, hearts were arrested by global ischemia for 30 min; therefore whole ventricle was considered as the area of risk. Normalized infarct size in % (Infarct size/area of risk) in the control heart subjected to ischemia and reperfusion was 33.43% (Figure 2, top). Percent of infarct size was significantly reduced for tomato juice group by 24.2% as compared with the control group and with lycopene the reduction is not significant, the value is 32.3%.

Cardiomyocyte apoptosis

We performed double antibody staining using antibody in Apop Tag kit and the monoclonal antibody recognizing cardiac myosin heavy chain to specifically identify cardiomyocyte apoptosis. A significant number of apoptotic myocytes was visible in the normal hearts subjected to 30 min ischemia and 2 h of reperfusion (22.0 ± 3.1%) (Figure 2, bottom). The number of apoptotic cells expressed as a percent of total cardiomyocyte population was significantly reduced in the hearts that were pretreated with lycopene (15.0 ± 2.7%) or tomato juice (9.0 ± 1.5%) compared to control hearts.

Effects of tomato juice and lycopene on ROS activity

The amount of ROS activity in the heart was determined by examining the extent of lipid peroxidation by measuring MDA formation (Figure 3). The amount of MDA was about 274 pmol/gm of the heart after ischemia and reperfusion. This amount was reduced by 51% with tomato juice while lycopene decreased it to 37%.

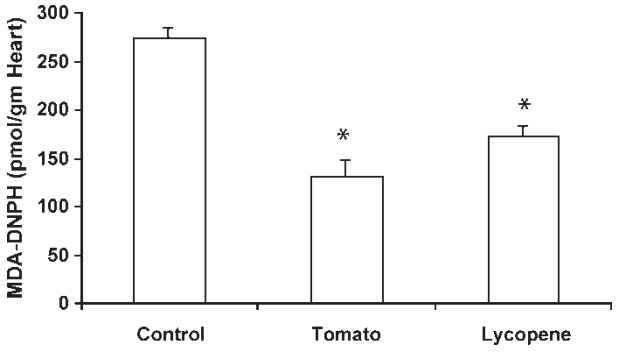

Figure 3. Effects of tomato juice and lycopene on the malonaldehyde content of the heart at the end of 30 min ischemia and 2 h of reperfusion. Results are expressed as Means ± SEM of six hearts per group. *$p < 0.05$ vs. control.

Discussion

The salient features of this study are (i) although both tomato juice and lycopene reduced the oxidative stress in the heart, only tomato juice, but not lycopene, was able to improve post-ischemic ventricular function; (ii) only tomato juice reduced myocardial infarct size and (iii) tomato juice, but not lycopene reduced apoptotic cell death; and (iv) tomato juice, but not lycopene, resulted in cardiac hypertrophy. These results clearly demonstrate that lycopene does not possess any cardioprotective role against myocardial ischemia/reperfusion injury. In contrast, tomato juice can render the heart resistant to ischemia/reperfusion injury.

Epidemiological studies indicate that regular consumption of tomatoes lower the risk of cancer and heart disease. *In vivo* studies have shown tumor-suppressive activity of lycopene as evidenced by reduction of mammary tumors in SHN virgin mice fed a diet enriched in lycopene [11]. However, lycopene failed to inhibit breast cancer, ovarian cancer or lung cancer [12–14]. In case of heart, lycopene lowered the oxidation of LDL-cholesterol and accounted in part for the lower risk of cardiovasvular disease [15]. The active antioxidant of tomato is lycopene, which is released from the tomato membrane and fiber during cooking. Thus, more lycopene becomes available from the processed tomatoes. Lycopene is a naturally occurring antioxidant consisting of an open-chain hydrocarbon structure containing 11 conjugated and two non-conjugated double bonds [16]. Lycopene is a potent singlet oxygen scavenger among the natural carotenoids scavenger and can reduce mutagenesis in the Ames test [17]. Lycopene also possesses antiproliferative and prodifferentiation activities [18]. Tomatoes also contain lycopene cyclase, an enzyme that can catalyze the conversion of lycopene beta-carotene

Despite of its antioxidant properties, the results of the present study showed that lycopene was unable to reduce the extent of myocardial ischemia/reperfusion injury. These results are inconsistent with many previous studies, which also did not find cardioprotection with lycopene. For example, dietary enrichment of endothelial cell with lycopene was unable to inhibit LDL oxidation [19]. Epidemiological evidence, however, shows antioxidant action of lycopene. For example, when normal human volunteers consumed a lycopene-free diet for 2 weeks, their serum lycopene levels went down and the amount of lipid peroxidation products increased [20]. Consistent with these results, when healthy volunteers consumed lycopene for one week, their serum lycopene levels went up and amount of lipid, protein and DNA oxidation products went down [21].

Health benefits of tomato consumption have become evident from several case-control studies. For example, anti-cancer effects of tomato consumption have been confirmed from epidemiological studies [22]. Fresh tomatoes contain many other antioxidants in addition to lycopene. Recent studies revealed that tomatoes contain significant amount of folic acid (13 μg/100 gm), vitamin A (74 IU/100 gm), vitamin C (23 mg/100 gm), β-carotene (0.2 mg/100 gm), vitamin E (0.32 mg/100 gm), quercetin (0.8 mg/100 gm) [23,24]. Investigation of the free radical scavenging activity and the antioxidant content of fresh and air-dried tomatoes revealed that tomato extracts could scavenge ROS and reduce lipid peroxidation [25]. Air-dried tomato extracts also possessed ROS scavenging activities, but partially lost their antioxidant reserve.

Epidemiological evidence exists to support the notion that regular consumption of tomatoes lower the risk factors for cancer and cardiovascular diseases. For example, the risk of prostate cancer was found to be significantly lower in men consuming five or more servings of tomatoes per week compared with men who consumed less than one serving of tomatoes per week [26]. A health professionals follow-up study (HPFS) also revealed a powerful linkage between consumption of tomato products and a lower risk of prostate cancer [22]. Although, case control studies were undertaken to evaluate cardiovascular benefits with lycopene consumption, no such reports are available for whole tomato consumption. To the best of our knowledge, this is the first report to show that tomato juice, but not lycopene, can reduce the cellular injury associated with myocardial ischemia and reperfusion. Our results suggest that hypertrophic response may play a crucial role in the cardioprotection achieved by tomato. It is not clear from this study whether antioxidants other than lycopene or hypertrophic response or both together are responsible for superior (compared to lycopene) cardioprotection. However, it was interesting to note that tomato results in hypertrophic response, and it is cardioprotective. The precise mechanism (hypertrophy vs. antioxidants) was not delineated in this study.

Acknowledgements

This study was supported by NIH HL 34360, HL 22559 and HL 33889.

References

[1] Rouse B, Matalon R, Koch R, Azen C, Levy H, Hanley W, Trefz F, de la Cruz F. Maternal phenylketonuria syndrome: Congenital heart defects, microcephaly, and developmental outcomes. J Pediatr 136:57–61.

[2] Halliwell B, Murcia MA, Chirico S, Aruma OI. Free radicals and antioxidants in food and *in vivo*: What they do and how they work. Crit Rev Food Sci Nutr 35:7–20.

[3] Das DK, Sato M, Ray PS, Maulik G, Engelman RM, Bertelli AA, Bertelli A. Cardioprotection of red wine: Role of polyphenolic antioxidants. Drugs Exp Clin Res 25:115–120, Review.

[4] Ray PS, Maulik G, Cordis GA, Bertelli AA, Bertelli A, Das DK. The red wine antioxidant resveratrol protects isolated rat

hearts from ischemia reperfusion injury. Free Radic Biol Med 27:160–169.
[5] Sato M, Maulik N, Das DK. Cardioprotection with alcohol: Role of both alcohol and polyphenolic antioxidants. Ann N Y Acad Sci 957:122–135.
[6] Nelson JL, Bernstein PS, Schmidt MC, Von Tress MS, Askew EW. Dietary modification and moderate antioxidant supplementation differentially affect serum carotenoids, antioxidant levels and markers of oxidative stress in older humans. J Nutr 133:3117–3123.
[7] Barr J, White WS, Chen L, Bae H, Rodermel S. The GHOST terminal oxidase regulates developmental programming in tomato fruit. Plant Cell Environ 27:840.
[8] Sato M, Cordis GA, Maulik N, Das DK. SAPKs regulation of ischemic preconditioning. Am J Physiol 279:H901–H907.
[9] Engelman DT, Watanabe M, Engelman RM, Rousou JA, Kisin E, Kagan VE, Maulik N, Das DK. Hypoxic preconditioning preserves antioxidant reserve in the working rat heart. Cardiovasc Res 29:133–140.
[10] Hattori R, Maulik N, Otani H, Zhu L, Cordis G, Engelman RM, Siddiqui MA, Das DK. Role of STAT3 in ischemic preconditioning. J Mol Cell Cardiol 33:1929–1936.
[11] Nagasawa H, Mitamura T, Sakamoto S, Yamamoto K. Effects of lycopene on spontaneous mammary tumor development in SHN virgin mice. Anticancer Res 15:1173–1178.
[12] Jarvinen R, Knekt P, Seppanen R, Teppo L. Diet and breast cancer risk in a cohort of Finnish women. Cancer Lett 114:21–23.
[13] Helzlsouer KJ, Alberg AJ, Norkus EP, Morris JS, Hoffman SC, Comstock GW. Prospective study of serum micronutrients and ovarian cancer. J Natl Cancer Inst 88:32–37.
[14] Steinmetz KA, Potter JD, Folsom AR. Vegetables, fruit and lung cancer in the Iowa women's health study. Cancer Res 53:536–543.
[15] Diaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. N Engl J Med 337:408–416.
[16] Britton G. Structure and properties of carotenoids in relation to function. FASEB J 9:1551–1558.
[17] DiMascio P, Kaiser S, Sies H. Lycopene as the most effective biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538.
[18] Amir H, Karas M, Giat J, Danilenko M, Levy R, Yermiahu T, Levy J, Sharoni Y. Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemia cells. Nutr Cancer 33:105–112.
[19] Dugas TR, Morel DW, Harrison EH. Dietary supplementation with β-carotene, but not with lycopene inhibits endothelial cell-mediated oxidation of low-density lipoprotein. Free Radic Biol Med 26:1238–1244.
[20] Rao AV, Agarwal S. Effect of diet and smoking on serum lycopene and lipid peroxidation. Nutr Res 18:713–721.
[21] Rao AV, Agarwal S. Bioavailability and *in vivo* antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. Nutr Cancer 31:199–203.
[22] Giovannuci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in risk of prostate cancer. J Natl Cancer Inst 87:1767–1776.
[23] US Department of Agriculture. Agricultural research service, USDA nutrient database for standard reference 1997, http://http://www.nal.usda.gov/fnic/foodcomp/ (release 11-1).
[24] Beecher GR. Nutrient content of tomatoes and tomato products P.S.E.B.M., vol 218.
[25] Lavelli V, Hippeli S, Peri C, Elstner EF. Evaluation of radical scavenging activity of fresh and air-dried tomatoes by three model reactions. J Agric Food Chem 47:3826–3831.
[26] Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 64:598–604.