Protective Effect of Dietary Tomato against Endothelial Dysfunction in Hypercholesterolemic Mice

Hiroyuki SUGANUMA and Takahiro INAKUMA

Research Institute, Kagome Co. Ltd., Nishitomiyama-17, Nishinasuno-machi, Nasu-gun, Tochigi 329-2762, Japan

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The effects of dietary ingestion of tomato were studied in mice that had been made hypercholesterolemic by feeding atherogenic diets. Mice which had been fed on the atherogenic diet without tomato for 4 months had significantly increased plasma lipid peroxide, and the vaso-relaxing activity in the aorta induced by acetylcholine (ACh) was harmed when compared with mice fed on a common commercial diet. On the other hand, mice which had been fed on the atherogenic diet containing 20% (w/w) lyophilized powder of tomato showed less increase in the plasma lipid peroxide level, and ACh-induced vaso-relaxation was maintained at the same level as that in normal mice. These results indicate that tomato has a preventive effect on atherosclerosis by protecting plasma lipids from oxidation.

Key words: atherosclerosis; endothelium; lipid peroxide; tomato

Atherosclerosis is a principal factor of many diseases such as cerebral apoplexy and myocardial infarction. Oxidative modification of plasma low-density lipoprotein (LDL) has been suggested to play a significant role in atherogenesis by facilitating the accumulation of lipids in macrophages in vitro and ex vivo.1,2 Endothelium-dependent relaxation evoked by ACh has been inhibited by treating oxidized LDL3,5 in vitro and was markedly reduced in atherosclerotic arteries.6 Endothelium-dependent vascular relaxation may thus be an index of atherosclerotic lesions. Carotenoids, which are widely distributed in fruits and vegetables, can act as antioxidants by quenching singlet oxygen (\(\text{O}_2^+\))7,8 and scavenging free radicals.9,10 They exist within LDL particles in the blood vessels and may participate in the protection of LDL from oxidative modification leading to the foam cell.11 Tomato includes many carotenoids such as lycopene and \(\beta\)-carotene, and continual ingestion of tomato juice significantly elevated the plasma concentration of those carotenoids.12 Consequently, the dietary uptake of tomato can be expected to prevent atherosclerosis by protecting LDL from oxidative modification. Yamaguchi et al.13,14 have established an animal model for primary screening of antiatherosclerotic agents with antioxidative activity by dietary linoleic acid in cholesterol-fed mice. With this model, the serum lipid peroxides were closely related to the enhancement of aortic cholesterol deposition, and the endothelium-dependent relaxation in the aorta might be reduced.

In the present work, we confirm that the uptake of an atherogenic diet harmed the function of the endothelium, and we assess the effect of dietary tomato on atherogenic diet-induced hypercholesterolemic mice by measuring the endothelium-dependent vaso-relaxation evoked by ACh and the endothelium-independent vaso-contraction induced by norepinephrine (NE) in thoracic aortae.

Materials and Methods

Materials. All materials for the diets were purchased from Oriental Yeast (Tokyo, Japan), except for the powdered tomato. The lyophilized powder of tomato used in this study was obtained from concentrated tomato juice supplied by local distributors. Acetylcholine chloride (Daiichi Pharmaceutical, Tokyo, Japan), dl-norepinephrine (Sankyo, Tokyo, Japan), and (R)-(--) phenylephrine hydrochloride and papaverine hydrochloride (Wako Pure Chemical Ind., Osaka, Japan) were used.

Animals and diets. Male mice of the ICR strain (Charles River Japan, Tokyo, Japan) and 4 weeks old were used as the experimental animals. The mice were fed on a stock diet (type CE-2, Japan CLEA, Tokyo, Japan) for 2 weeks, and were then assigned to 3 groups. The mice in group A received the atherogenic diet without tomato, those in group B were given the atherogenic diet containing 20% (w/w) powdered tomato, and the mice in group C were fed on a common commercial diet (OYC modified AIN-93G, Oriental Yeast, Tokyo, Japan). All animals were allowed free access to water and the respective diet for 4 months in a temperature (24-26°C) and humidity (40-60%) controlled room with a 12 h cycle of light (06:00-18:00 h) and dark. The body weight of each animal was measured daily. The lyophilized powder of concentrated tomato juice was supplemented to the diet, the composition of the atherogenic diet with tomato being as follows (g/100 g): casein, 20.0; sucrose, 31.3; agar, 2.0; coconut oil, 10.0; linoleic acid, 10.0; AIN-93G mineral mixture, 3.5; AIN-93 vitamin mixture, 1.0; cholesterol, 1.5; cholic acid, 0.5; choline bitartrate, 0.2; and powdered tomato, 20.0. In the atherogenic diet without tomato, to mimic the

1 To whom correspondence should be addressed. Fax: +81-287-39-1038; E-mail: Hiroyuki_Suganuma@kagome.co.jp

Abbreviations: LDL, low-density lipoprotein; ACh, acetylcholine; TBARS, thiobarbituric acid-reactive substance; NE, norepinephrine; PE, phenylephrine

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composition of the tomato powder,10 20 g/100 g of powdered tomato was replaced as follows: casein, 3.52; glucose, 5.26; fructose, 5.26; cellulose, 2.50; and corn starch, 3.46. The blood of each mouse was drawn from the orbital sinus by two micro-hematocrit tubes at intervals of 2 months from the start of the feeding experiment. The blood in one tube (20 μl) was infused in 1 ml of physiological saline and then centrifuged. The lipid peroxide level of the supernatant was measured. The other tube was placed to clot the blood and then centrifuged. The resulting supernatant was used for measuring the serum total cholesterol. After feeding the experimental diets for 4 months, the animals were killed by decapitation to obtain the thoracic aortae.

Biochemical analyses. The concentrations of serum total cholesterol and lipid peroxide were measured with commercial kits (Cholesterol E-Test and Lipid Peroxide-Test; Wako Pure Chemical Ind.). Lipid peroxides were fluorometrically determined by the thiobarbituric acid method of Yagi16 and their levels are expressed as the concentration of malondialdehyde which reacted with thiobarbituric acid. Each isolated artery was cut into about a 4-mm length of ring and placed in a 5-ml tissue organ bath (UC-5, Kishimoto Medical Ind., Kyoto, Japan) containing a physiological salt solution with the following composition (mm): NaCl, 120; KCl, 4.7; MgSO4, 1.2; KH2PO4, 1.2; CaCl2, 2.5; NaHCO3, 25; and glucose, 10. The solution was maintained at 37±0.5°C and bubbled with 95% O2 and 5% CO2 (pH 7.4) throughout the experiments. The relaxation and contraction of each aorta preparation were detected with a force-displacement transducer (IM-20BS, Star Medical, Tokyo, Japan) connected to a computer system (Eight Star, Star Medical) with which isometric tension changes were recorded and memorized. The resting tension was adjusted to 0.5 g. The preparations were allowed to equilibrate for > 60 min in the medium, during which time the solution was replaced every 10 min. The endothelium-dependent relaxation was assessed by the cumulative addition of ACh to a pre-contracted aorta preparation with 1 μM phenylephrine. The maximal relaxation induced by 10 μM papaverine is taken as 100%, and the ED50 value is expressed as the ACh concentration required to trigger 50% relaxation. The vasocontractile activity was examined by the cumulative addition of NE.

Statistical analysis. Each data value is expressed as the mean and SE. Values were subjected to an analysis of variance, and differences between means were considered significant at p<0.05 by Tukey's test, including the cases with unequal sample sizes.17

Results

Figure 1 shows the changes in body weight throughout the feeding experiment. The mean body weight gains (±SE) of groups A, B and C were 11.5±1.2, 11.1±0.7 and 18.0±2.4 (g), respectively. Groups A and B, which were fed on the atherogenic diets, were sig-
significantly inferior in growth to group C. Among the two groups that received the atherogenic diets, the presence or absence of powdered tomato didn't affect the growth of the mice.

The changes in the serum total cholesterol and plasma TBARS levels are shown in Figs. 2a and 2b). Serum total cholesterol in groups A and B, which were fed on the atherogenic diets, was significantly increased compared with group C. The plasma TBARS levels increased in all groups as the feeding experiment advanced, the increase in group A being larger than that in the other groups. Group B, which was supplemented with tomato, also received the atherogenic diet, but the increase in plasma TBARS level was only slight and similar to that of group C which was fed on a common commercial diet.

The endothelium-dependent relaxation evoked by ACh was compared in the 3 experimental groups on thoracic aortae (Figs. 3 and 4). The ACh-induced relaxation in group A was significantly inferior to that in the other two groups. Consequently, the mean ED30 values for groups A, B and C were 4.50 ± 0.92, 1.75 ± 0.34 and 1.93 ± 0.45 (nm), respectively, there being a statistically significant difference between group A and the others (Tukey's test, \( p < 0.05 \)). Continuous intake of the atherogenic diet harmed the endothelium-dependent vasorelaxation of thoracic aortae, and supplementing the powdered tomato in the diet prevented this impairment.

We then assessed the endothelium-independent contraction induced by NE of the same aortae to clarify the mechanism by which tomato protected the endothelial dysfunction (Fig. 5). The contraction of group B tended to be stronger than that of the others, but was not statistically significant. The contractions evoked by 60 mM KCl in groups A, B and C were 0.99 ± 0.06, 1.05 ± 0.06 and 0.98 ± 0.06 (g), respectively, there being no significant difference.

Discussion

We found in the present study that the dietary ingestion of tomato prevented an increase in plasma TBARS and protected from a dysfunction of the endothelium in mice made hypercholesterolemic by feeding the atherogenic diet.

In the mouse model for hypercholesterolemia used in this study, the plasma level of TBARS was markedly increased and oxidatively modified LDL is considered to
have caused atherosclerotic lesions. The intake of the atherogenic diet without tomato harmed the endothelium-dependent relaxation induced by ACh, but not the endothelium-independent contraction of vascular smooth muscle evoked by KCl or NE. Consequently, in this study, the function of the endothelium was impaired before that in the smooth muscle. This dysfunction of the endothelial cells is thought to have been the incipient stage of atherosclerosis and was prevented by supplementing the atherogenic diet with powdered tomato. Yokoyama et al.\textsuperscript{3,4} have reported that oxidatively modified LDL inhibited the ACh-induced endothelium-dependent relaxation of rabbit aorta but not the nitroglycerin-induced endothelium-independent relaxation. This agrees with our results.

Tomato includes a relatively large amount of lycopene, one of the antioxidative carotenoids, and the powdered tomato used in this experiment contained 0.16% lycopene. It has been reported that the serum level of lycopene was increased by continual uptake of tomato juice in humans.\textsuperscript{12,18} After absorption, lycopene exists mainly within LDL particles in human plasma. Oshima et al.\textsuperscript{19} have indicated that supplementation with lycopene inhibited the singlet oxygen-mediated oxidation of human plasma LDL. In our study, the uptake of tomato inhibited the increase of lipid peroxide in mouse plasma. This suggests that lycopene might act as an antioxidant in plasma and that it protected plasma lipoprotein from oxidative modification. We didn’t measure the level of lycopene in this study, and carotenoids are thought to be less absorbable by rodents than by humans. However, dietary lycopene has been found to protect against the development of spontaneous mammary tumors in SHN virgin mice\textsuperscript{20} and against the formation of colonic aberrant crypt foci in rats.\textsuperscript{21} So lycopene may be absorbable by rodents and act as an antioxidant. We thus consider that the dietary ingestion of tomato inhibited the oxidative modification of serum lipids and consequently prevented the dysfunction of the endothelium.

In conclusion, two things are indicated by the results of this study. First, dietary ingestion of tomato inhibited the increase of lipid peroxide in mouse plasma. Second, it prevented the impairment of ACh-induced relaxation of mouse aorta produced by the continuous intake of an atherogenic diet. It is thought that these beneficial effects of tomato were caused by lycopene acting as an antioxidant. These findings strongly suggest that tomato would exert a preventive effect on atherosclerosis mediated by oxidatively modified lipoprotein. This study was made to assess the preventive effects of tomato as a natural food against atherosclerosis. It remains to be elucidated whether the dietary ingestion of lycopene would exert the same effects as tomato.

References
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Fig. 5. Aortic vaso-contraction Induced by the Cumulative Addition of NE in Mice Fed on Various Diets.

NE-induced contraction of mice aortae in group A (○, n = 10), group B (●, n = 10) and group C (△, n = 7) are shown. The maximal contraction induced by 60 mM KCl is taken as 100%. Each point is the mean, and a vertical bar indicates SE.


