Beneficial Effect of Tomato Juice Drinking on Anti-Mutagenicity of Saliva

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Abstract

Objectives: The purpose of this study was to investigate the effect of tomato juice drinking on the anti-mutagenicity of saliva.

Methods: Subjects were 22 healthy male university students. They were divided into tomato group and control group. The tomato group drank tomato juice for 10 days. The anti-mutagenicity of saliva was measured using the umu test.

Results: In the tomato group, there was a significant increase in the inhibiting capacity of saliva on the mutagenicity of AF-2 after tomato juice drinking for 10 days. This increase was, however, temporary. In the control group, there was no such change in the inhibiting capacity of saliva.

Conclusions: These findings suggest the significant effect of tomato juice drinking on the anti-mutagenicity of saliva. In addition, lycopene may have played an important role in its mechanism.

Key words: anti-mutagenicity, human saliva, umu test, tomato juice drinking, lycopene

Introduction

In our previous study, we found a relation between lifestyle and anti-mutagenicity of saliva (1). Namely, the inhibiting capacity of saliva on the mutagenicity of AF-2 was higher in those who had many poor habits. These findings appeared to be related to their greater intake of mutagens. On the other hand, a “nutritionally balanced diet” was the only lifestyle item that tended to contribute positively to the inhibiting capacity of saliva. Thus, we considered that intake of some nutrients may be related to the inhibiting capacity of saliva.

Meanwhile, a protective effect of tomato against carcinogenesis is suggested. Epidemiological studies have revealed an inverse relation between tomato intake or serum lycopene level and the risk for several types of human cancers (2, 3). In addition, lycopene, a carotenoid abundantly present in tomato, has been shown to possess strong antioxidant capability (4, 5). Thus, in this study, we investigated the effect of 100% pure tomato juice drinking on the anti-mutagenicity of saliva.

Materials and Methods

Subjects and protocol

Subjects were 22 healthy male university students (mean age;

23.0±2.6 years). All individuals were nonsmokers and did not take any drugs. These subjects were divided into two groups, tomato juice drinking group (tomato group) and control group, at random. Basic characteristics of each group are shown in Table 1. There was no significant difference in age, physical characteristics or lifestyle (Health Practice Index (6–8)) between the two groups. In addition, a questionnaire revealed that there was no significant difference in their daily intake of vegetables or vegetable juice. The tomato group drank 3 cans (570 g) per day (morning, noon, night) of tomato juice (Yakult Co., Tokyo) for 10 days.

Saliva sampling was carried out on three different days: immediately before beginning tomato juice drinking, immediately after finishing tomato juice drinking (10 days after) and 3 days after finishing tomato juice drinking. Saliva samples of 10–15 ml were collected in test tubes directly (9). All samples were collected at the same time (17:00) to exclude the effect of possible circadian variation. It has been reported that many components of saliva were stable in the mid-afternoon (10). Subjects were requested to refrain from food and beverages for at least 2 hours before each saliva sampling and not to consume similar beverages during the study period. The samples were stored at –80°C until the assay.

Anti-mutagenicity assay

The anti-mutagenicity of saliva was measured using the umu test. Furfuryluramide (AF-2) of 0.1 ml (0.024 µg/ml) was used as a mutagen. The mutagenicity of AF-2 has been well confirmed by the umu test (11–13).

Bacteria were grown in either Luria's broth or TGA medium (1%-bacto tryptone, 0.5%-NaCl and 0.2%-glucose) supplemented with ampicillin (20 mg/ml). Z-buffer was prepared as described by

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Table 1 Characteristics of subjects

Group	Number of subjects	Values (mean±SD)				
		Age (yr.)	Height (cm)	Body weight (kg)	BMI (kg/m ²)	Health Practice Index
Tomato group	11	23.5±3.6	172.2±4.1	60.6±4.6	20.4±1.2	5.1±0.9
Control group	11	22.6±1.0	172.7±4.5	62.7±8.3	21.0±1.9	4.5±1.1

Miller (14). Chemicals were of the purest grade available.

The umu test was essentially carried out as described previously (15–17) using a tester strain, *Salmonella typhimurium* TA1535/pSK1002. Saliva of 0.2 ml was added to the test system. It was reported that this quantity of saliva sufficiently inhibits the mutagenicity of AF-2 (11). In addition, in the pretest, there was no effect of saliva on the proliferation of the tester strain. The SOS responses were measured as the β-galactosidase activity by the method of Miller (14). The SOS responses-inhibiting capacity of saliva (%) was obtained using the following equation (18):

$$\text{The SOS responses-inhibiting capacity} = [1 - (A - C) / (B - D)] \times 100;$$

where, A is the β-galactosidase activity induced by the mutagen mixed with saliva, B is that by the mutagen, C is that by saliva, and D is that by no addition (baseline).

Statistical analysis

All values were expressed as means±SD. ANOVA with repeated measures was performed to detect between-group and time-related differences. Bonferroni's test was used for multiple comparisons. Values were considered to be significantly different if $p < 0.05$.

Results

The modifying effect of saliva on the mutagenicity of AF-2 is shown in Table 2. In the present study, there was an inhibiting capacity of $33.3 \pm 25.5\%$.

The effect of tomato juice drinking on the inhibiting capacity of saliva is shown in Table 3. In the tomato group, there was a significant increase in the inhibiting capacity of saliva immediately after finishing tomato juice drinking ($p < 0.05$). This increase was, however, temporary. Namely, the inhibiting capacity of saliva showed a tendency to return to the baseline level 3 days after

Table 2 Modifying effect of saliva on SOS responses induced by AF-2

	β-galactosidase activity (units)		Inhibiting capacity (%)
	AF-2 (0.024 μg/ml)	DMSO	
Saliva (+)	584.05±165.99	171.29±28.64	33.3±25.5
Saliva (−)	829.53±15.52	210.65±5.64	

Values are expressed as means±SD.

Number of saliva donors was 22.

finishing tomato juice drinking. Meanwhile, in the control group, there was no significant change in the inhibiting capacity of saliva during the study period.

Discussion

Tomato juice used in the present study included lycopene of 19 mg per can (190 g) as an anti-mutagen. Thus, it may have mainly contributed to the inhibiting capacity of saliva.

It has been shown that active oxygen species such as singlet oxygen or free radicals can cause a wide spectrum of cell damage including lipid peroxidation, enzyme inactivation and DNA damage (19–22). Namely, the generation of active oxygen species may play an important role in carcinogenesis. The conjugated polyene structure of carotenoids is involved in quenching such harmful active oxygen species (23). Actually, many antioxidants are anti-promoters and anticarcinogens (20). In addition, it was reported that lycopene possesses exceptionally high antioxidant capability compared with other carotenoids (4).

The effect of other substances in tomato juice, however, remains to be taken into account. To confirm the effect of lycopene itself, therefore, further studies using supplemental lycopene are in the planning stage. In addition, measurement of salivary lycopene levels may also become a clue to solving this problem.

In the tomato group, there was a significant increase in the inhibiting capacity of saliva immediately after finishing tomato juice drinking. This increase, however, did not continue to 3 days after finishing tomato juice drinking. These findings suggest that the effect of tomato juice drinking on the inhibiting capacity of saliva is immediate but not persistent. To obtain a persistent effect, therefore, it appears that tomato juice should be consumed regularly.

The level/quantity of lycopene/tomato that is beneficial has not yet been clearly assessed (3). In addition, there is little information on the absorption, distribution and metabolism of lycopene (3). In the present study, subjects drank 3 cans per day of tomato juice for 10 days, because we considered that this quantity may have a sufficient effect on the inhibiting capacity of saliva and can be taken by subjects without strain. As a result, according to our expectations, there was the significant effect of tomato juice drinking. In future studies, the relation between intake of tomato juice and inhibiting capacity of saliva should be further clarified.

In conclusion, the present findings support our hypothesis that intake of some nutrients is related to the inhibiting capacity of saliva. It is expected that similar studies will be carried out for

Table 3 Effect of tomato juice drinking on the inhibiting capacity of saliva

Inhibiting capacity (%)			
	Immediately before	Immediately after	3 days after
Tomato group (n=11)	29.8±28.0	37.8±28.6*	35.9±25.4
Control group (n=11)	31.0±27.3	32.9±23.5	32.5±25.0

Tomato juice drinking was performed 3 times (190 g×3) a day for 10 days. Total amount of tomato juice drinking was 5,700 g.

Values are expressed as means±SD.

Significantly different from "immediately before beginning tomato juice drinking", * $p < 0.05$ (Repeated Measures ANOVA and Bonferroni's test).

other various nutrients.

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