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Original Article

Daily Lifestyles and Anti-Mutagenicity of Saliva

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Abstract

Objectives: The purpose of this study was to investigate the relation between lifestyle and the anti-mutagenicity of saliva.

Methods: Subjects were 52 healthy female university students. The collection of the saliva samples and the lifestyle measurements were carried out for them. The anti-mutagenicity of the saliva was measured using the umu test.

Results: With regard to the lifestyle items, only "nutrient balance" tended to contribute positively to the inhibiting capacity of the saliva on the mutagenicity of AF-2. In addition, there was a significant inverse correlation between the score of 7 other items and the inhibiting capacity of the saliva ($r=-0.32$; $p<0.05$). We also found a significant relation between their tea and/or coffee consumption and the inhibiting capacity of the saliva.

Conclusions: These findings suggest that the inhibiting capacity of saliva worked to decrease mutagen levels that were enhanced by poor lifestyle. In addition, "nutrient balance" may contribute to the inhibiting capacity of the saliva independent of 7 other items. With regard to the tea and/or coffee consumption, further studies should be carried out.

Key words: anti-mutagenicity, human saliva, umu test, lifestyle, tea and coffee consumption

Introduction

It is known that saliva has various functions such as digestive or oral environment-maintaining functions[1)]. Some previous studies found an inhibiting capacity of human saliva on mutagens[2–5)]. It may be very important to clarify the mechanism of the anti-mutagenicity of saliva, because saliva reacts first with mutagens in food.

Meanwhile, previous studies found mutagenicity in saliva of individuals who used some luxury goods such as chewing tobacco[6,7)] and the anti-mutagenicity in some foods such as tea or vegetables[5,8)]. Therefore, the condition of the saliva may be greatly related to lifestyle. In this study, we investigated the relation between lifestyle and the anti-mutagenicity of saliva and tried to clarify its mechanism.

Materials and Methods

Subjects were 52 healthy female university students. The collection of saliva samples and the lifestyle measurements (Health Practice Index; HPI[9–11)]) were carried out for them. Their basic characteristics are shown in Table 1.

Data collections were made after a lecture in a room. The saliva samples were collected in the test tubes directly for 2 minutes. All samples were collected at the same time (17:30) in order to exclude the effect of possible circadian variation. Ferguson et al.[12)] showed that many components of saliva were stable in the mid-afternoon. The samples were stored at −80°C until the assay.

The anti-mutagenicity of the saliva was measured using the umu test. Furylfuramide (AF-2) of 0.1 ml (0.024 μg/ml) was used as a mutagen. The mutagenicity of AF-2 was previously confirmed by the umu test[4,13,14)].

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 Miller[15)]. Chemicals were of the purest grade available.

Essentially, the umu-test was carried out as described

Table 1 Characteristics of subjects (n=52)

Age (yr.)	21.2±1.8
Height (cm)	158.3±4.9
Body weight (kg)	50.5±5.9
Health practice index	4.5±1.5

Values are expressed as means±SD.

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previously[16–18] using a tester strain, *Salmonella typhimurium* TA1535/pSK1002. The saliva of 0.2 ml was added. Okada et al.[4] reported that this quantity of saliva sufficiently inhibits the mutagenicity of AF-2. In addition, in the pretest, there was no effect of the saliva on the proliferation of the tester strain. The SOS responses were measured as the β-galactosidase activity by the method of Miller[15]. The SOS responses-inhibiting capacity of the saliva (%) was obtained using the following equation[19]:

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

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

All values were expressed as means±SD. Pearson's correlation coefficients were used to examine the relation between the variables. In addition, the one-way analysis of variance was used for the comparison among groups. 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. There was no significant correlation between the inhibiting capacity of saliva and the HPI score (Fig. 1). In addition, no lifestyle item contributed significantly to the inhibiting capacity of saliva (Table 3).

Particularly with regard to "working hours" and "physical exercise", however, the inhibiting capacity of saliva tended to be higher in those who had poor habits (Table 3). In addition, only "nutrient balance" tended to contribute positively to the inhibiting capacity of saliva.

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

	β-galactosidase activity (units)		Inhibiting capacity (%)
	AF-2 (0.024 μg/ml)	DMSO (control)	
Saliva (+)	835.64±169.25	207.37±41.37	20.2±19.2
Saliva (−)	1,017.52±29.75	230.14±27.40	

Values are expressed as means±SD.

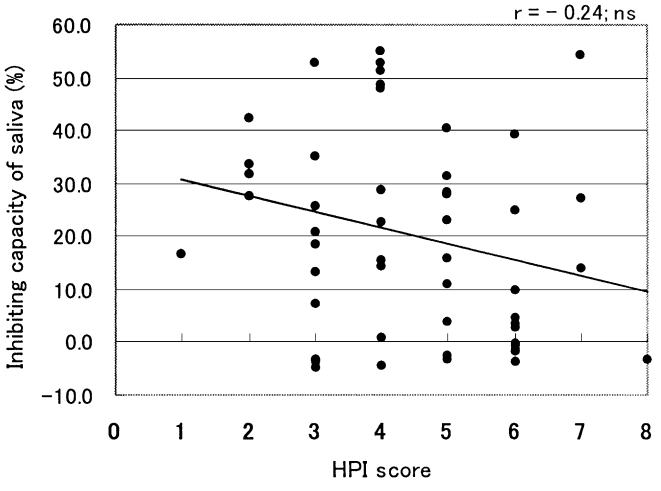

Fig. 1 HPI score and inhibiting capacity of saliva (n=52).

When divided into three groups according to the score of the 7 lifestyle items except for "nutrient balance", there was a significant difference in the inhibiting capacity of saliva among them ($p<0.05$). Multiple comparisons revealed a difference between the good and the poor lifestyle group (9.75±16.99% vs. 31.13±8.54%; $p<0.05$) (Fig. 2).

Meanwhile, the inhibiting capacity of the saliva was significantly lower among those who drank more than 5 cups of tea and/or coffee per day compared with those who did not drink (8.64±15.72 vs. 31.86±17.36; $p<0.05$) (Fig. 3).

Table 3 Lifestyle and inhibiting capacity of saliva (%)

Lifestyle	Good		Poor		* p value
Cigarette smoking	19.53±19.56	n=48	28.40±14.17	n=4	0.38
Consuming alcohol	20.02±19.52	n=50	25.15±11.81	n=2	0.72
Eating breakfast	19.77±20.15	n=42	22.08±15.54	n=10	0.74
Sleeping hours	17.39±18.39	n=18	21.71±19.77	n=34	0.45
Working hours	14.43±18.88	n=23	24.80±18.56	n=29	0.05
Physical exercise	13.60±17.74	n=19	24.02±19.28	n=33	0.06
Nutrient balance	28.66±21.18	n=10	18.20±18.44	n=42	0.12
Mental stress	16.82±17.71	n=22	22.70±20.21	n=30	0.28

Values are expressed as means±SD.
* Student's *t* test.

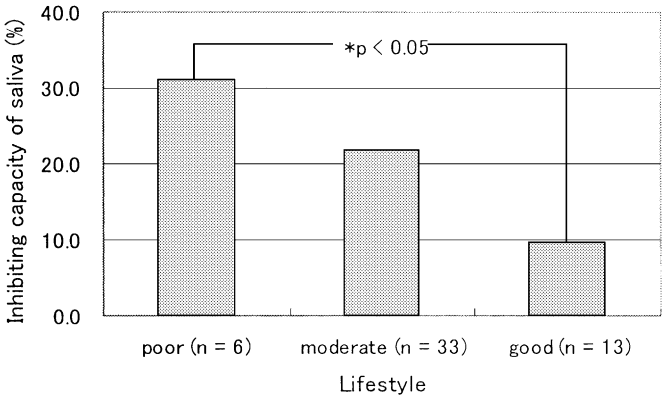

Fig. 2 Lifestyle and inhibiting capacity of saliva.
*** one-way analysis of variance and Bonferroni's test**
Lifestyle is defined as "poor" when the score of the 7 lifestyle items except for "nutrient balance" is 0–2, "moderate" when it is 3–5 and "good" when it is 6–7.

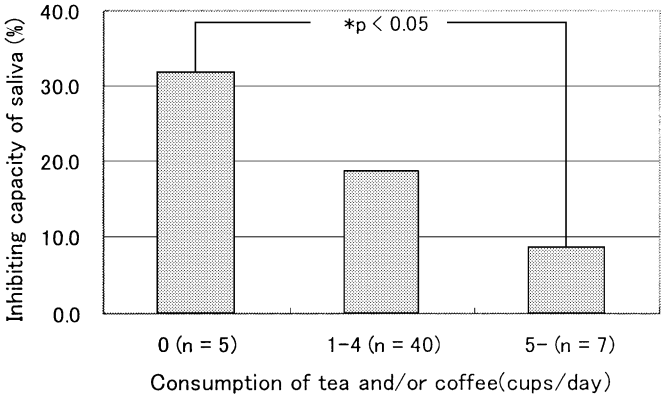

Fig. 3 Consumption of tea and/or coffee and inhibiting capacity of saliva.
*** one-way analysis of variance and Bonferroni's test**

Discussion

At present, the Ames test[20] and umu test are widely used as the method for the mutagenicity assay. In this study, the umu test was used because of many advantages, when compared with the Ames test; i) the sensitivities are similar, ii) only one bacterial tester strain is required for various types of mutation, iii) the assay is carried out in a shorter time and iv) it is not influenced by histidine. Thus, the umu test may be more suitable for large groups or samples that can include histidine such as components of the human body.

With regard to the 7 lifestyle items except for "nutrient balance", particularly "working hours" and "physical exercise", the inhibiting capacity of saliva tended to be higher in those who had poor habits (Table 3). In short, only "nutrient balance" tended to contribute positively to the inhibiting capacity of saliva. As a result, there was a significant inverse correlation between the score of these 7 items except for "nutrient balance" and the inhibiting capacity of saliva ($r=-0.32$; $p<0.05$) (Fig. 4). These findings might lead to the following hypothesis: Those who have poor lifestyles tend to intake more mutagens in their daily life compared with those who have good lifestyles, and the inhibiting capacity of the saliva works to decrease enhanced mutagen levels in such individuals. In short, a balance is maintained between the intake of mutagens and the inhibiting capacity of the saliva. Some previous studies on the HPI[21,22] indicated that poor lifestyles increase the urinary levels of mutagens. These findings support the above stated hypothesis.

"Nutrient balance" may, however, contribute to the inhibiting capacity of the saliva independent of such a mechanism. Although the mechanisms of the anti-mutagenicity of saliva are not yet fully understood, previous studies[2,3] suggested that some substances may be related to the inhibiting capacity of saliva (Table 4).

As described above, "physical exercise" and "working hours" were markedly related to the inhibiting capacity of saliva (Table 3). In a previous study of judo players[23], we also found a decrease in the inhibiting capacity of saliva in non-weight reduction control subjects. They were first-rate athletes preparing for a competition, and needed to intake a considerable amount of energy to adapt to hard training. In fact, the tendency to take unlimited amounts of various foods was widely observed among them. Therefore, it was suggested that such a confusion of the energy intake might contribute to the decrease in the inhibiting capacity of the saliva. However, the present subjects were not athletes and did not show the tendency to intake food excessively. To clarify the relation between the inhibiting capacity of saliva and physical exercise, therefore, further studies should be carried out.

In addition, the inhibiting capacity of saliva was higher among those who worked greater than 10 hours. Since all the present subjects were students, however, we considered school and part-time job hours as "working hours". To confirm the present findings, therefore, further studies should be carried out for workers.

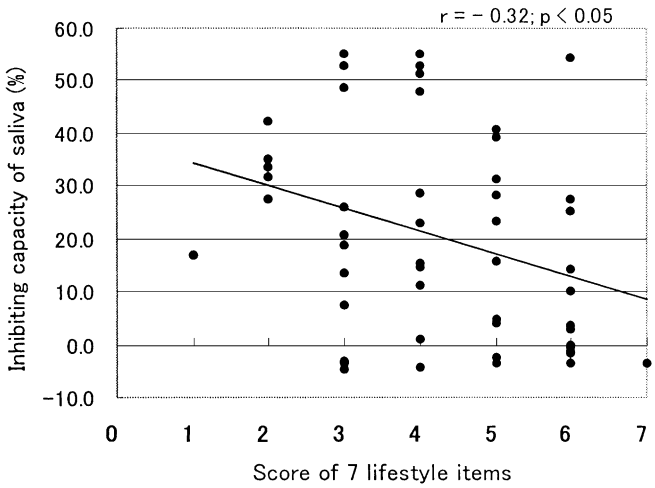

Fig. 4 Score of 7 lifestyle items except for "nutrient balance" and inhibiting capacity of saliva (n=52).

Table 4 Substances that can be related to the inhibiting capacity of saliva

Lower molecular weight substances	Enzymes
	Vitamins
	Hormones
	Amino acids
	Metal ions
	Other organic and inorganic substances
Higher molecular weight substances	Bacteria
	Viruses
	Proteins
	Secretions from mucous membranes
	Dental calculus
	Blood plasma
	Others

Meanwhile, the salivary secretion rate of the present subjects was 0.50±0.21 ml/min. This agreed with the findings of Navazesh et al.[24]. However, the salivary secretion rate was not significantly correlated with the inhibiting capacity of saliva. Therefore, the salivary secretion rate may not have influenced the anti-mutagenicity of saliva.

In addition, the present subjects were all females. Therefore, the effects from menstrual cycle could not be avoided. Since all females had different timing of the cycle, however, this does not appear to have contributed to the results in a significant way.

We also investigated their tea and/or coffee consumption. As a result, the inhibiting capacity of saliva was significantly lower among those who drank more than 5 cups/day compared with those who did not drink (8.64±15.72 vs. 31.86±17.36; $p<0.05$) (Fig. 3). Stich et al.[5] found the anti-mutagenicity of Japanese, Chinese and Ceylonese tea. However, they also found that saliva lost its inhibiting capacity when mixed with Chinese tea, and this supports our findings. In the present study, the total consumption of tea and/or coffee was asked. In future studies, therefore, the consumption of each item should be investigated.

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