A Further Note on the Sampling Device for the Anti-Mutagenicity of Saliva

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Abstract

Objectives: The purpose of this study was to compare the anti-mutagenicity of Salivette and test-tube sampling saliva. In addition, the relation between the inhibiting and pH-buffering capacities of saliva was investigated.

Methods: Subjects were 52 healthy female university students. The collection of saliva samples was carried out using 2 sampling devices; test-tube and Salivette. The anti-mutagenicity of the saliva was measured using the umu test.

Results: The inhibiting capacity of Salivette-saliva was significantly lower compared with that of test-tube-saliva ($p < 0.01$, t test). However, there was a significant correlation between them ($r = 0.35$; $p < 0.05$). In addition, there was a significant correlation between the inhibiting and pH-buffering capacities of saliva ($r = -0.36$; $p < 0.05$).

Conclusions: These findings suggest that both the Salivette and the test-tube may be appropriate as saliva-sampling devices. In addition, they suggest that the bicarbonates might inhibit the anti-mutagenicity of saliva, or that the activity of substances related to the anti-mutagenicity of saliva might be dependent on pH.

Key words: anti-mutagenicity, human saliva, umu test, saliva sampling device, buffering capacity

Introduction

It may be very important to clarify the anti-mutagenicity mechanism of saliva, because saliva reacts first with mutagens in food. In our previous study¹⁾, we investigated the relation between lifestyle and the anti-mutagenicity of saliva using the umu test. However, investigations from a different viewpoint may also need to be carried out regarding the anti-mutagenicity of saliva. Thus, we investigated the relation between the anti-mutagenicity and the buffering capacity of saliva as an additional study. The buffering capacity of saliva is related to salivary pH and is very important from the viewpoint of the prevention of oral caries^{2,3)}.

In addition, the selection of the saliva-sampling device is an important problem, because it has not been well established for the umu test. Thus, we compared 2 sampling devices; test-tube and Salivette.

Materials and Methods

Subjects were 52 healthy female university students (mean

age; 21.2 ± 1.8 years). The saliva samples were collected first in the test-tubes directly for 2 minutes, and then using Salivette® (Sarstedt Co. Ltd., Nümbrecht), a device to obtain saliva by centrifuging (at 3,000 rpm for 15 min) from cotton that subjects have chewed (for 2 min). All samples were collected at the same time (17:30) to exclude any effect of possible circadian variation. The samples were stored at -80°C until the assay.

The anti-mutagenicity of the saliva was measured using the umu test⁴⁻⁶⁾. Furfuryluramide (AF-2) of 0.1 ml (0.024 $\mu\text{g/ml}$) was used as a mutagen. The mutagenicity of AF-2 was previously confirmed by the umu test⁷⁻⁹⁾. Then, 0.2 ml of saliva was added as described by Okada et al.⁷⁾. The SOS responses were measured as the β -galactosidase activity by the method of Miller¹⁰⁾. The SOS responses-inhibiting capacity of saliva (%) was obtained using the following equation¹¹⁾:

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

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).

The buffering capacity of the saliva was measured using test-tube-saliva as described by Ericsson²⁾, and expressed as final pH.

All values were expressed as means $\pm$ SD. Student's t test was used for the comparison between groups. In addition, Pearson's correlation coefficients were used to examine the relation between the variables. Values were considered to be significantly different if $p < 0.05$.

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Table 1 Modifying effect of human saliva on SOS responses induced by AF-2

	β -galactosidase activity (units)		Inhibiting capacity (%)
	AF-2 (0.024 μ g/ml)	DMSO (control)	
Test-tube-saliva	835.64 $\pm$ 169.25	207.37 $\pm$ 41.37	20.2 $\pm$ 19.2
Salivette-saliva	988.89 $\pm$ 100.75	213.43 $\pm$ 34.36	1.5 $\pm$ 10.9*
Saliva(-)	1,017.52 $\pm$ 29.75	230.14 $\pm$ 27.40	

Values are expressed as means $\pm$ SD.

* Significantly different from test-tube-saliva, $p < 0.01$ (Student's t test)

Table 2 Comparison between PBS through and not through the cotton swabs of Salivette

	β -galactosidase activity (units)	
	AF-2 (0.024 μ g/ml)	DMSO (control)
PBS (through c. swabs)	1,036.43 $\pm$ 22.85	260.43 $\pm$ 0.35
PBS (not through c. swabs)	1,056.19 $\pm$ 34.26	268.81 $\pm$ 23.33

Values are expressed as means $\pm$ SD.

Results and Discussion

The modifying effects of test-tube- and Salivette-saliva on the mutagenicity of AF-2 are shown in Table 1. The inhibiting capacity of Salivette-saliva was significantly lower compared with that of test-tube-saliva (1.5 $\pm$ 10.9 vs. 20.2 $\pm$ 19.2; $p < 0.01$). Although the mechanism of the anti-mutagenicity of the saliva is not yet known, previous studies have suggested that both higher and lower molecular weight substances are associated with it^(7,12). In short, its mechanism may include biochemical reactions with enzymes, vitamins, etc. and biophysical adsorption by proteins, bacterial cells, mucous materials, etc.^(12,13) Therefore, the present findings may suggest that almost all the higher and some lower molecular weight substances adhered to cotton swabs of Salivette. In addition, there was no significant difference in the results of the umu test between PBS through and not through the cotton swabs of Salivette (Table 2). These findings suggest that the cotton swab itself did not influence the results.

In addition, there was a significant correlation between the inhibiting capacities of test-tube- and Salivette-saliva ($r = 0.35$; $p < 0.05$) (Fig. 1). These findings suggest that lower molecular weight substances may contribute to the inhibiting capacity of the saliva on the mutagenicity of AF-2 at a constant ratio. In other words, the trend of lower molecular weight substances may reflect the whole trend. Thus, both the test-tube and the Salivette may be appropriate as a saliva-sampling device. When using Salivette, however, devices to examine the lower inhibiting capacity, e.g. more sample addition to the test system, may be required.

The buffering capacity of saliva is mainly determined by the bicarbonate concentration (HCO_3^-) and is related to the prevention of oral caries^(2,3). By neutralizing acid, the bicarbonates inhibit the growth of acidophilic bacteria such as Streptococci or Lactobacilli.

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Fig. 1 Correlation between the inhibiting capacities of Salivette and test-tube sampling saliva **Fig. 2** Correlation between the buffering and inhibiting capacities of saliva

In this study, the buffering capacity of saliva was 3.90 ± 0.80 . It was significantly related to the inhibiting capacity of test-tube-saliva ($r = -0.36$; $p < 0.05$) (Fig. 2). These findings might lead to the following hypotheses; i) the bicarbonates inhibit the anti-mutagenicity of saliva and ii) the activity of substances concerning the anti-mutagenicity of saliva is dependent on pH.

As described above, some substances in saliva may be related to its anti-mutagenicity. Among them, oral bacteria or dental calculus are generally more active under acid conditions⁽³⁾. In addition, the salivary peroxidase system is promoted under acid conditions⁽³⁾. These findings support our hypothesis-ii. With regard to the relation between the anti-mutagenicity of saliva and the bicarbonates itself (hypothesis-i), however, further studies should be carried out.

In addition, some previous studies^(2,14,15) suggested that an increased intake of vegetables and fruits is positively related to a higher buffering capacity. In future studies, therefore, research considering the dietary habit should be carried out.

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