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Original Research

Evaluation of environmental change in the mouth with the use of spray-type oral moisturizer containing γ-PGA

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ABSTRACT

Objective: This study was to evaluate the effect of new spray-type oral moisturizer containing poly-γ-glutamic acid (γ-PGA). γ-PGA can be classified as a pseudopolyamino acid, which contains only repeated glutamate units. The hygroscopic and moisturizing effects of γ-PGA were compared with HA. Cross-linked γ-PGA could absorb thousands of times more water than its own weight.
Methods: 102 volunteers were randomly allocated to experimental and control groups. The experimental group (50 subjects) used this new moisturizer, the control group I (20 subjects) used sterile distilled water at normal temperature, and the control group II (32 subjects) used nothing. In experimental group, the effect of moisturizer was investigated by measuring amylase activity (salivary amylase monitor®), oral moisture (Mucus®), stimulated salivary flow rates at predetermine times (Saxon test).
Results: In the amylase activity and the oral moisture, there was not a significant difference in all groups. In the stimulated salivary flow rates, there was a significant difference. When we compared the experimental group with the control group I, an increase was admitted at after 30 min ($p < 0.05$). When we compared the experimental group with the control group II, an increase was admitted at after 10 ($p < 0.01$), 20 ($p < 0.05$), 30 min ($p < 0.05$).
Conclusions: New moisturizer was able to improve the environment in the mouth. Oral application of γ-PGA has a possibility to be a therapeutic strategy for treating Xerostomia.
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1. Introduction

Saliva is an important body fluid that plays a vital role in protecting the oral mucosa and teeth, as well as aiding oral function and the digestion of food. An absence of saliva results in a number of oral changes and related behaviors that can influence a patient's quality of life [1–5].

Xerostomia induces bacteria propagation in the oral environment making it vulnerable to pneumonia caused by aspiration of saliva. Treatment includes reduction of stress and drug treatment such as herbal medicine and salivary gland hormones. Systemic therapies include taste stimulus treatment [6], massage of salivary glands [7], gum treatment [8], and using a moisturizing agent [9–11].

In Japan, Aquabalance® medical mouthspray was commercialized as the first spray-type oral moisturizer containing γ-PGA by LION Dental Products Company in 2013.

Poly-γ-glutamic acid (γ-PGA) can be classified as a pseudopolyamino acid, which contains only repeated glutamate units. However, γ-PGA differs from proteins, both structurally and functionally. The glutamate units in γ-PGA are linked between the α-amino and γ-carboxylic acid functional groups, whereas the peptide linkages in proteins are formed between the amino and γ-carboxylic acid groups. Bacillus subtilis (natto) was isolated from the traditional Japanese food, natto, Fermented soybean health food containing abundant γ-PGA, which is popular in Japan. Forming a highly mucous colony, the strain can grow on a high-saline medium containing L-glutamic acid. Bacillus subtilis (natto) is a plasmid-free γ-PGA producer [12].

The purpose of this study is analysis of the oral environmental changes with this spray.

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2. Materials and methods

2.1. Subjects

The subjects were 102 healthy Japanese students ranging from 20 to 28 years old (46 males and 56 females) at Osaka Dental University. All subjects gave written informed consent to participation in this study after receiving explanation of its purpose. The experimental group was 50 subjects (27 males and 23 females) who had Aquabalance medical mouthspray® sprayed on the back of the tongue. The control group I was 20 subjects (5 males and 15 females) who had sprayed sterile distilled water at normal temperature on the back of the tongue. The control group II was 32 subjects (14 males and 18 females) who did not receive spray application.

2.2. Oral moisturizer

Aquabalance medical mouthspray® (AB) used in this study is a spray-type oral moisturizer containing l-menthol, γ-poly glutamic acid (Bacillus natto gum) [13], glycerin, propylene glycol, polyoxyethylene hydrogenated castor oil (60E.O.), xylitol, buffering agent, flavor, and cetylpyridinium chloride.

2.3. Study design

This study was undertaken with the approval of the Institutional Review Board (IRB) of the Osaka Dental University Hospital (IRB number: 110837).

2.3.1. Examination 1: Measurement of amylase activity by salivary amylase monitor

Amylase was measured with a salivary amylase monitor (Nipro Co., Japan) and test strip (Nipro Co., Japan) [14,15]. We used this measurement method because it was easy and less invasive, since the test strip was placed under the tongue for only 30 s and also because it only required about 1 min from saliva sampling until obtaining the result.

The salivary amylase monitor builds on a precursor colorimetric assay platform wherein the color intensity of the enzymatic reaction product is photometrically measured to determine the concentration of salivary α-amylase. The biosensor system comprises of a disposable test strip and a hand-held reader with digital display. The test strip integrates a collector pad at its tip and a reagent paper infused with Gal-G2-CNP, a chromogenic substrate for salivary α-amylase. When placed under the tongue, the collector pad quickly saturates with fixed micro-liter quantities of saliva. The strip is inserted into the reader and the lever rose to activate the reader and transfer the collected saliva onto the reagent paper. After a 10 s interval, an audible signal indicates completion of saliva transfer and the strip is retracted. The salivary amylase activity in the transferred saliva begins metabolizing the Gal-G2-CNP substrate to yield the colored product CNP according to the reaction. Enzymatic activity is allowed to proceed for 10 s and the reflectance of the reaction product then measured photometrically at 430 nm. The color measured is proportional to the concentration of the salivary amylase activity; the greater the intensity of the color observed, the greater the concentration of salivary amylase activity. The biosensor's microprocessing unit calculates the salivary amylase activity level and displays it as a digital readout along with a date and time stamp [16,17].

Table 1
Measuring of amylase activity by salivary amylase monitor.

Group of subjects	Experimental	Control I		Control II	
	Mean SD	Mean SD	*p*-value	Mean SD	*p*-value
Baseline	21.68	37.42		26.02	
After 10 min	20.17	38.79	NS	24.67	NS
After 20 min	24.67	36.56	NS	25.21	NS
After 30 min	18.73	35.94	NS	24.74	NS

2.3.2. Examination 2: Measurement of oral moisture by Mucus® [18]

Oral moisture was measured at the lingual mucosa (10 mm from the apex linguae on the surface of the tongue) and at the right and left buccal mucosa (10 mm from the angle of the mouth).

2.3.3. Examination 3: Measurement of stimulated salivary flow rates by Saxon test [19]

Saliva production was measured by weighing a folded sterile gauze pad before and after chewing. The low-normal value is 2 g/2 min.

The subjects were randomly allocated to one of the three groups:

Experimental group: 50 persons who used AB.
Control group I: 20 persons who did nothing.
Control group II: 32 persons who used sterile distilled water.

We performed all examinations in baseline (before application them) after 10, 20, and 30 min for experimental and control group.

We carried out this study between noon and 3 pm and measured after more than 1 h of eating and/or drinking. During the experiment, eating, drinking, and conversation were forbidden. The subjects were told to relax in a sitting position.

2.4. Statistical analysis

We compared the experimental group with the control group I and II with *T*-test. We compared baseline after 10, 20, and 30 min on each group.

3. Results

3.1. Examination 1

We measured amylase activity by salivary amylase monitor.

There was not a significant difference between Experimental group and Control group I for 30 min.

There was not a significant difference *between Experimental group and Control group II* for 30 min (Table 1).

3.2. Examination 2

We measured oral moisture by Mucus.

There was not a significant difference between Experimental group and Control group I for 30 min.

Table 2
Measuring of the oral moisture by Mucus®.

Group of subjects	Experimental	Control 1		Control 2	
	Mean SD	Mean SD	*p*-value	Mean SD	*p*-value
Baseline	1.95	2.2		1.7	
After 10 min	2.69	1.65	NS	1.72	NS
After 20 min	2.3	1.7	NS	1.2	NS
After 30 min	1.99	1.85	NS	1.85	NS

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