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JNMU
Journal of Nanjing Medical University, 2009, 23(1):69-72
www.elsevier.com/locate/jnmu
Research paper

The Antibacterial Effect of CMCTS-Containing Chewing Gum

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Received 27 October 2008

Abstract

Objective: This paper was designed to confirm the efficacy of chewing carboxymethyl chitosan(CMCTS)-containing gum in suppressing the growth of oral bacteria when compared to a CMCTS-containing mouth rinse. **Methods:**Fourteen healthy subjects were recruited from among the staff and students of Qingdao University Dentistry Department. Before the experiments saliva was collected from all subjects and bacteria counts determined. For the gum study, the subjects chewed CMCTS-containing gum for 5 min and then rested for 5 min. When testing the CMCTS mouth rinse, the subjects gargled with 10 mL of solution for 30 s, followed by resting for 9min 30 s. These protocols were repeated five times over a 50 min period on the same day. Post-experiment saliva samples were then collected at the following times: 0, 30 and 60 min. **Results:** Chewing gum containing CMCTS or rinsing with a CMCTS-containing rinse significantly decreased oral bacteria counts. The total bacteria counts, total Streptococci counts, and mutans streptococci counts of saliva from subjects who chewed CMCTS-containing gum were significantly lower than saliva from subjects in the rinse group in all three sampling periods, except in the case of the total bacteria count in the 60 min samples. **Conclusion:**CMCTS-containing gum chewing has a greater antibacterial effect than using a CMCTS-containing mouth rinse. The present findings strongly indicate that the application of natural materials such as chitosan and its derivatives is useful for better oral health.

Key words: Carboxymethyl chitosan; Chewing gum; Mouth rinse; Cariogenic bacteria

INTRODUCTION

Chitosan is a cationic natural polymer derived from chitin which has significant biomedical potential. It consists of 2-acet-amido-2-desoxy-β-D-glucopyranose and de-acetylated 2-amino-2-desoxy-β-D-glucopyranose monomers, with the amount of deacetylated monomers exceeding the acetylated ones. Being a weak base, chitosan is soluble in a weak acidic environment, but insoluble at physiological or higher pH values, whereas chitosan derivatives offer better solubility at near-neutral pH values[1]. Carboxymethyl chitosan(CMCTS) is a modified chitosan that has good solubility in aqueous media.

Gum chewing is thought to be a mechanically effective method for cleaning teeth, second only to tooth brushing. The addition of antibiotics to chewing gum also improves the antibacterial effect. However, antibiotics have several adverse effects, such as the occurrence of anaphylaxis, vomiting, diarrhea, bacterial resistance and tooth staining[2].

Natural bioactive materials have recently been investigated as promising agents to prevent oral diseases such as dental caries. CMCTS is a biocompatible and biodegradable material. It is positively charged and combines with the bacterial cell wall and membrane with bacteriostatic and bactericidal results[3-6]. Water-soluble, reduced chitosan was used as a mouth rinse reagent, and it displayed an antibacterial and plaque-reducing action[7,8]. Furthermore, recent studies demonstrated that chewing chitosan-containing gum effectively inhibited the growth of cariogenic bacteria(total bacteria, total Streptococci, mutans streptococci(MS)) in saliva[9]. Gum chewing is reported to remove an average of 80% of

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residual oral debris[10]. However, there are no studies in which the antibacterial effects of CMCTS-containing gum were compared to those of a CMCTS-containing mouth rinse. The present study was designed to determine whether chewing CMCTS-containing gum more effectively suppressed the growth of oral bacteria than a mouth rinse using a CMCTS solution.

MATERIALS AND METHODS

Subject recruitment

Eight healthy adults(4 males and 4 females) ranging in age from 20 to 22 years(a mean age: 21.1) and eight healthy adults(4 males and 4 females) ranging in age from 27 to 42 years(a mean age: 30.1) were recruited for the antibacterial activity experiment. All volunteers were non-smokers and had at least 28 natural teeth with no current caries activity, periodontal disease, or other oral pathology. None of the subjects were using antibiotics or medications.

Study protocols

The xylitol-based chewing gum(slab type, 2.5 g per piece) used for the in situ studies was supplemented with CMCTS. The CMCTS oligomer added to the chewing gum was soluble. The adding of chitosan is about 1% in the gum. For each study, all subjects crossed over to each randomly assigned treatment, with at least 1 week between treatments. For 1 week before the experiment and the 1 week rest period between treatments, all subjects were instructed to use a dentifrice without fluoride.

The subjects brushed their teeth immediately after lunch. Two hours later, following a 5-min period of wax mastication, a pre-experimental saliva sample was collected in a 50 mL centrifuge tube. The gum chewing stage involved chewing one piece of gum for 5min, followed by a rest for 5 min. The CMCTS mouth rinse solution involved gargling with 10 mL of solution for 30 s, followed by a rest for 9 min 30 s. This protocol was repeated five times over a 50 min period on the same day.

After five periods of gum chewing, or five rinses with CMCTS solution, post-experimental saliva was collected at the following times: 0, 30 and 60 min.

Culturing of samples

After vortexing for 2 min, paraffin-stimulated saliva samples were homogenized by ultra-sonication under ice-cold water twice for 15 s at 4℃. Because the ultra-sonicatio is carried in ice-cold water at low temperature in a short time, we think the ultra-sonicatio has little affect to the colony counts. Serial 10-fold dilutions of the suspensions were prepared in a BHI(Brain Heart Infusion) medium(BBL Microbiology Systems, Cockeysville, MD, USA). Aliquots of the appropriate dilutions were plated in triplicate on sheep blood agar for total oral bacteria, on Mitis-salivarius agar for total Streptococci, and on Mitis-salivarius agar containing 0.2 U/mL bacitracin for MS. The plates were incubated anaerobically(BD GasPackTM, MD, USA) at 37℃ for 48 h. The colony numbers were counted and then the results of triplicate samples were averaged.

Statistical analysis

The colony counts of post-treatment saliva samples obtained at the three different time periods were converted to percentages of the pre-treatment saliva counts from, the same individual. A two-way analysis of variance(ANOVA) of repeated measures was used to compare the growth of bacteria in the two treatment groups for 1 h after gum chewing or rinsing. The statistical difference between the groups at each sampling time was assessed using an independent t-test.

RESULTS

The original CFU/mL data are presented in ***Table 1*** and ***Table 2***. These data showed that there was considerable variation in the counts, particularly of MS cultures. However, all the CMCTS groups showed inhibition of the bacterial counts. The relative percentage (CFU/mL) was calculated and analyzed in ***Table 3***.

ANOVA analyses indicated that CMCTS-containing gum chewing significantly decreased the growth level of bacteria($P < 0.005$) in comparison to the mouth rinse group, despite the high variability and large standard deviations, particularly in total bacteria counts. The percentage decrease in all three parameters(total bacteria, total Streptococci, MS) in the gum group was significantly greater($P < 0.05$) than in the rinse group during all three sampling times, except at 60 min in the case of the total bacteria count. The difference between the gum group and the rinse group was larger for total Streptococci than for other examined bacteria. Although the decreases in the counts for all three parameters were the greatest at 0 min in the gum group, in the rinse group the decreases were greatest at 30 min.

DISCUSSION

The antibacterial effect of a mouth rinse using a CMCTS solution was compared with that of chewing a CMCTS-containing gum. Since a mouth rinse is the simplest and most convenient method for oral hygiene, this method is the first choice when testing the oral antimicrobial activity of a compound. Furthermore, the cost of production is lower for the mouth rinse. The only problem with a CMCTS-containing mouth rinse is the taste. Subjects may complain of the astringent and/or bitter taste of CMCTS. It is important when supplementing with CMCTS oligomer to use a xylitol-containing chewing gum, because xylitol could mask

Table 1 **Colony counts(CFU/mL) in saliva samples related to rinse**

	Rinse group(min)			
	Before Rinsing	0	30	60
Total bacteria				
Mean	3.62 ± 10^8	2.65 ± 10^8	2.35 ± 10^8	
Median	2.89 ± 10^8	2.48 ± 10^8	1.34 ± 10^8	3.33 ± 10^8
Minimum	0.98 ± 10^8	0.67 ± 10^8	0.43 ± 10^8	1.21 ± 10^8
Maximum	7.99 ± 10^8	4.89 ± 10^8	3.51 ± 10^8	0.93 ± 10^8
Total streptococci				4.01 ± 10^8
Mean	5.89 ± 10^7	4.95 ± 10^7	3.42 ± 10^7	
Median	2.98 ± 10^7	1.78 ± 10^7	1.66 ± 10^7	3.29 ± 10^7
Minimum	0.90 ± 10^7	0.81 ± 10^7	0.65 ± 10^7	2.21 ± 10^7
Maximum	13.93 ± 10^7	9.38 ± 10^7	8.02 ± 10^7	0.90 ± 10^7
Mutans streptococci				5.89 ± 10^7
Mean	5.28 ± 10^5	2.35 ± 10^5	1.74 ± 10^5	2.13 ± 10^5
Median	0.11 ± 10^5	0.09 ± 10^5	0.06 ± 10^5	0.12 ± 10^5
Minimum	0.24 ± 10^3	0.15 ± 10^3	0.10 ± 10^3	0.17 ± 10^3
Maximum	21.78 ± 10^5	10.12 ± 10^5	9.17 ± 10^5	9.49 ± 10^5

Table 2 **Colony counts(CFU/mL) in saliva samples related to chewing**

	Gum group(min)			
	Before Rinsing	0	30	60
Total bacteria				
Mean	4.24 ± 10^8	0.76 ± 10^8	1.15 ± 10^8	0.83 ± 10^8
Median	2.08 ± 10^8	0.94 ± 10^8	1.44 ± 10^8	0.61 ± 10^8
Minimum	0.78 ± 10^8	0.67 ± 10^8	0.43 ± 10^8	0.93 ± 10^8
Maximum	10.61 ± 10^8	1.65 ± 10^8	2.11 ± 10^8	1.79 ± 10^8
Total streptococci				
Mean	6.53 ± 10^7	1.05 ± 10^7	1.30 ± 10^7	1.43 ± 10^7
Median	3.78 ± 10^7	1.23 ± 10^7	1.32 ± 10^7	1.33 ± 10^7
Minimum	0.59 ± 10^7	0.15 ± 10^7	0.16 ± 10^7	0.19 ± 10^7
Maximum	13.65 ± 10^7	2.48 ± 10^7	3.52 ± 10^7	3.01 ± 10^7
Mutans streptococci				
Mean	4.52 ± 10^5	0.85 ± 10^5	0.98 ± 10^5	0.63 ± 10^5
Median	0.51 ± 10^5	0.29 ± 10^5	0.36 ± 10^5	0.40 ± 10^5
Minimum	0.91 ± 10^3	0.25 ± 10^3	0.18 ± 10^3	0.22 ± 10^3
Maximum	19.88 ± 10^5	3.62 ± 10^5	3.17 ± 10^5	5.49 ± 10^5

Table 3 **Percentage of the bacteria count**

	Rinse group			Gum group		
	0 min	30 min	60 min	0 min	30 min	60 min
Mean total bacteria	$78.9 \pm 22.1\%$	$63.2 \pm 28.8\%$	$67.5 \pm 37.3\%$	$31.7 \pm 25.3\%$	$43.2 \pm 25.9\%$	$41.4 \pm 31.6\%$
Mean total streptococci	$85.8 \pm 19.5\%$	$68.5 \pm 11.9\%$	$76.7 \pm 29.6\%$	$28.4 \pm 13.5\%$	$33.1 \pm 15.8\%$	$37.9 \pm 24.1\%$
Mean mutans streptococci	$72.4 \pm 15.8\%$	$56.6 \pm 14.8\%$	$68.9 \pm 26.8\%$	$32.4 \pm 19.7\%$	$34.3 \pm 21.8\%$	$43.3 \pm 26.2\%$

the astringent and/or bitter taste of CMCTS.

There may be a major difference in the effective CMCTS concentration between the gum chewing and mouth rinse groups. Subjects did not rinse with tap water after rinsing in CMCTS. Supplements in chewing gum are usually released into saliva within 3 min[11]. Thus, after 5 min the saliva CMCTS concentration in the chewing gum group was not necessarily higher than that in the rinse group.

The time used in mouth rinsing in this case may have been too short when compared to gum chewing. However, a 30-s rinse is the maximum time for a single rinse due to the oral capacity for holding bubbles, so that continuous rinsing for 5 min is practically impossible. If continuous rinsing was possible with the administration of additional CMCTS solution in the mouth, then its concentration in the rinse experiment would have been much higher than in the gum chewing experiment.

The present data pose the question as to why the gum chewing was more effective in inhibiting the growth of oral bacteria than the mouth rinse. The gum chewing provides both the mechanical debridment of the teeth surface through mastication[12,13] and the direct antibacterial action of the released chitosan. Furthermore, gum chewing increases the flow rate of saliva which may

also have accelerated the clearance of cariogenic bacteria.

As we all know chewing gum accelerate salivary secretion. This effect could be useful for the maintenance of oral health, as well as aid in normalizing systemic well being. Adequate salivary flow is essential for a healthy oral environment. In older people, in particular, salivary flow rates are generally low and they experience xerostomia due to physiological or pathological conditions, and due to several commonly used medications[14]. One would therefore expect that a stimulus of salivary secretion provided by a natural and gentle substance, such as CMCTS, would be advantageous for the maintenance of health in the elderly. The supplementation of chewing gum with CMCTS is therefore considered desirable because the gum base is an effective vehicle for the continuous release of bioactive materials such as chitosan. Furthermore, the act of gum chewing has been linked to improved mental health, emotional stability, and stress relief, including after sleep deprivation, and results in increased salivary secretion[15-19].

Carboxymethyl chitosan-containing gum chewing had a greater antibacterial effect than a CMCTS-containing rinse. The present findings strongly indicate that the application of natural materials such as CMCTS in a vehicle such as chewing gum is useful for both oral health and improving the quality of life.

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