Dental Plaque pH Recovery Effect of Arginine Bicarbonate Rinse In Vivo

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Objective: *To investigate the neutralising effects of subsequent arginine bicarbonate rinse on sucrose-induced decrease in plaque pH with interdental plaque pH telemetry.*
Methods: *Six participants wearing partial lower prostheses incorporating a miniature glass pH electrode were included. After 5 or 6 days of plaque accumulation on the tip of the electrode, the subjects rinsed with a 15 ml 10% sucrose solution, followed no subsequent rinsing or rinsing with 15 ml of water, or 85 mmol/L $NaHCO_3$, or 0.5%, 1% or 2% arginine bicarbonate for 2 min. The plaque pH was continuously recorded for 120 min.*
Results: *Without a subsequent rinse, the plaque pH decreased at 10 min to 4.38 and stayed below the critical 5.7 for 83 min after sucrose rinse. Subsequent water rinse showed little effects on the sucrose-induced decrease in plaque pH and $NaHCO_3$ induced only an instantaneous pH rise, whereas subsequent arginine bicarbonate rinses all immediately and effectively neutralised the sucrose-induced decrease in plaque pH.*
Conclusion: *These results strongly suggested that regular use of an arginine bicarbonate rinse after carbohydrate consumption could help prevent caries.*

Key words: *plaque pH, caries prevention, sucrose; Stephan curve, arginine*

Dental plaque pH is one of the critical factors for initiation and progression of caries. Acid and base formation both occurs in dental plaque and counteracts each other in the determination of plaque pH. The acid-base cycling by the plaque bacteria stimulates the demineralisation–remineralisation cycling of the tooth beneath the plaque. Saliva provides significant benefits in maintaining and promoting healthy teeth. Saliva contains not only the buffers bicarbonate and phosphate that help neutralise the acids produced by the plaque bacteria, but also nitrogenous substrates, which contribute significantly to base formation, to counter the pH lowering or raise the pH of the plaque when it is producing or has produced acid from periodic dietary carbohydrate[1]. The nitrogenous substrates within the mouth that contribute significantly to base formation are urea and arginine.

Arginine was first isolated from saliva by Kleinberg[2], it attracts much more attention not only because it is a base forming substrate, but its continual presence favours the growth and metabolism of arginolytic bacteria[1]. Thus it may help to prevent the emergence of cariogenic microflora, and maintains a healthier, base producing plaque microflora[1,3]. There was a strong correlation between elevated levels of free arginine in saliva and caries resistance[4–6]. However, arginine is found in saliva in micro molar concentrations[4]. Arginine bicarbonate buffering salt has been used in oral care products to provide the base forming substrate and acid buffering. It has been successfully used in the treatment of dentinal hypersensitivity[7–9]. Recent clinical studies also demonstrated its caries preventive effect[10,11].

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The acid countering effect of arginine bicarbonate was supposed to be responsible for the caries inhibition. However, whether the antiacid effect of arginine bicarbonate was due to the base generation of arginine or the buffering activity of the bicarbonate remains to be fully tested *in vivo*.

The aim of this study was to examine *in vivo* the neutralising effects of various concentrations of arginine-bicarbonate solutions or the equivalent concentration of sodium bicarbonate solution on sucrose-induced decreases in pH of interproximal plaque using interdental plaque pH telemetry.

Materials and methods

Subjects

Six healthy subjects (4 males and 2 females) with a mean age of 63 years (56–72 years) and 2 or 3 missing teeth in the premolar and molar regions of the mandible were recruited from the Department of Prosthodontics, Peking University School and Hospital of Stomatology. All subjects had a stimulated salivary secretion rate > 0.6 ml/min, and the mean pH was 7.67. All subjects had no unfilled cavities, periodontal disease, or other oral diseases. Ethical approval for this study was obtained from the Ethical Committee of Peking University Health Science Centre, and written consent was obtained from all subjects.

Interdental plaque pH telemetry

Interdental plaque pH telemetry was performed as described in detail by Imfeld et al[9]. Briefly, for each subject a mandibular partial prosthesis was fabricated incorporating a miniature glass pH electrode (W. Möller). The tip of the electrode faced the interdental surface of the subject's adjacent abutment teeth below the proximal contact point. The pH was continuously recorded (μR 1000, Yokogawa) and the original pH curve was scanned (intuos3, VACOM) and analysed by the computer software (TelDat, Vesion 1.5, Boling). The electrode was calibrated with standard buffer pH 7.00 before each test session.

Plaque accumulation

The accumulation of plaque on the tip of the electrode was performed as described in detail previously[9]. Briefly, the subjects were asked to wear the prostheses with clean electrodes remaining in place, not to remove the dental device and not to alter their eating habits. They had to refrain from all oral hygiene measures for the entire experimental period, except for rinsing with water and tooth brushing without toothpastes. The pH measurement was performed in the early morning on the sixth or seventh day of plaque accumulation without having eaten or drunk anything except water before the test.

Experiment procedure

The experiment procedure was also performed as described in detail previously[9,10], but with minor modifications. The subjects were arranged to test one of the solutions each visit, with an interval of 1 week. Each subject attended the experiment on 6 occasions in total. The experiment started with the chewing of paraffin for 3 min for plaque-pH normalization. After an initial period of 20 min for establishment of baseline value, the subjects rinsed with 15 ml of 10% sucrose solution for 2 min; 10 min after expectoration, the subjects rinsed with 15 ml of tap water, 85 mmol/L sodium bicarbonate, 0.5%, 1%, and 2% arginine bicarbonate, respectively, for 2 min. The pH value was continuously recorded for 120 min for each test. A sucrose rinse without subsequent treatment was initially administered as a control for each subject. All arginine bicarbonate solutions and sodium bicarbonate solution were freshly prepared in distilled water before use. A total of 36 (6×6) telemetric curves were recorded. The pH values at 10 min after sucrose rinse, time for plaque pH curve below 5.7, area of plaque pH curve under 5.7 ($AUC_{5.7}$), area of plaque pH curve above 5.7 ($AAC_{5.7}$), and the highest pH after arginine bicarbonate rinse were calculated from the telemetric curves.

Acid buffer capacity test

The pH of 5 ml 85 mmol/L sodium bicarbonate or 5 ml 2% arginine bicarbonate was tested respectively, and then the volume of 0.107 N HCl to bring the pH to pH 7 was measured with automatic titration system (ZDJ-3D, Xianfeng, China).

Statistical analysis

Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc). All data were presented as means ± SD. Two-way analysis of variance (ANOVA) was performed for comparison of difference between groups. A value of $P < 0.05$ was considered statistically significant.

Results

Sucrose rinse resulted in prolonged decrease in plaque pH

Without subsequent treatment, the plaque pH decreased to 4.38 at 10 min after sucrose rinse and stayed below critical 5.7 for 83 min (Fig 1 and Table 1).

Water rinse failed to neutralise sucrose-induced prolonged decrease in plaque pH

To examine the effect of a water rinse on a sucrose-induced prolonged decrease in plaque pH, the water rinse was applied after the sucrose rinse. After the sucrose rinse, the plaque pH decreased to 4.45 at 10 min; after a subsequent water rinse, the plaque pH jumped close to 5.7 and fell back quickly and stayed below 5.7 for 75 min (Fig 2 and Table 1).

Sodium bicarbonate solution

To investigate the neutralising ability of bicarbonate, 85 mmol/L sodium bicarbonate solution (pH 7.88) was tested as a control for arginine bicarbonate. After a sucrose rinse, the plaque pH decreased to 4.32 at 10 min, whereas after a subsequent sodium bicarbonate rinse, the plaque pH sharply rose to 6.63 and soon dropped down below 5.7 for 49 min (Fig 3 and Table 1). The $AUC_{5.7}$ of sodium bicarbonate was significantly smaller than that of the water rinse, but larger than the 2% arginine bicarbonate rinse (Table 1).

Arginine bicarbonate rinse effectively neutralised sucrose-induced prolonged decrease in plaque pH

To examine the effect of an arginine bicarbonate rinse on a sucrose-induced decrease in plaque pH, 0.5%, 1%, and 2% arginine bicarbonate rinses were tested respectively after a sucrose rinse. The pH of the three solutions was almost the same (pH 8.53). After the sucrose rinse, the plaque pH decreased to the level similar to that of the water and control groups at 10 min, whereas after a subsequent arginine bicarbonate rinse, the plaque pH sharply rose to the highest level (around 7.0) and soon dropped down within a few minutes, but remained above the critical 5.7 until the end of the measurement (about 70 min) in all arginine bicarbonate groups (Fig 3 and Table 1). The times for plaque pH below pH 5.7 and $AUC_{5.7}$ of arginine bicarbonate groups were all significantly less than that of the water and control groups ($P < 0.01$). Although there was a trend that the higher the concentration of arginine bicarbonate rinse was, the

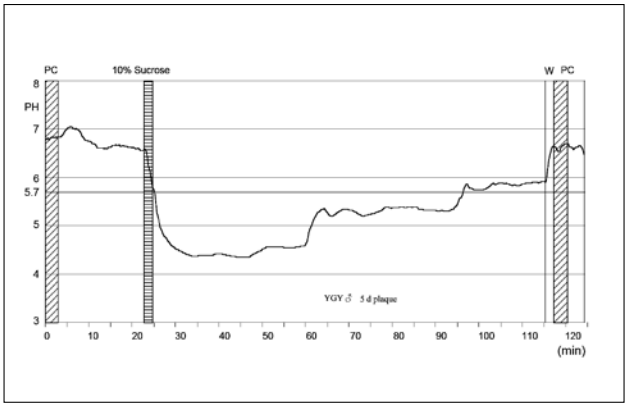

Fig 1 Telemetrically recorded interdental plaque pH profile of the subject YGY during and after sucrose rinse. W = water rinse, PC = paraffin chewing.

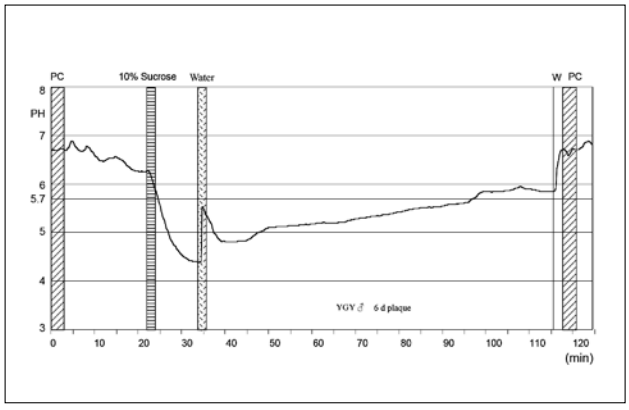

Fig 2 Telemetrically recorded interdental plaque pH profile of the same subject during and after a sucrose rinse and a subsequent water rinse. W = water rinse, PC = paraffin chewing.

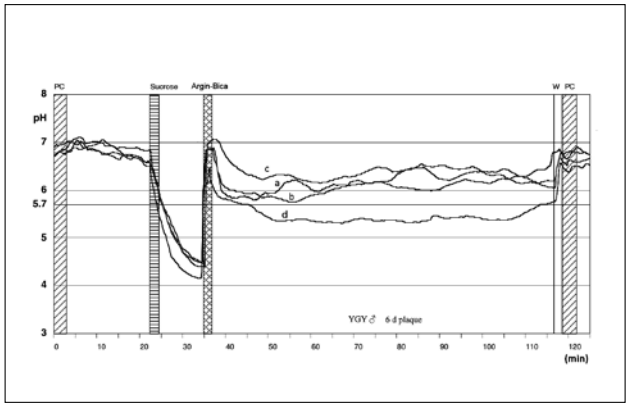

Fig 3 Telemetrically recorded interdental plaque pH profile of the same subject during and after a sucrose rinse and a subsequent 0.5% (a), 1% (b) and 2% (c) arginine bicarbonate rinse, and 85 mmol/L sodium bicarbonate (d). W = water rinse, PC = paraffin chewing.

Table 1 Data (mean ± SD) from plaque pH response curve to 10% sucrose rinse and subsequent water or arginine-bicarbonate (n = 6)

Groups	pH at 10 min after sucrose rinse	Time for plaque pH below pH 5.7	Highest pH after subsequent rinse	$AUC_{5.7}$ (arbitrary unit)	$AAC_{5.7}$ (arbitrary unit)
Sucrose	4.38 ± 0.19	83.00 ± 16.43	NA	79.29 ± 12.93	NA
Water	4.45 ± 0.15	75.40 ± 17.07	NA	35.09 ± 16.75	NA
$NaHCO_3$	4.32 ± 0.14	49.40 ± 13.92*	6.63 ± 0.43	13.53 ± 9.12*	9.17 ± 7.82
0.5% Arginine-Bicarbonate	4.41 ± 0.21	14.83 ± 18.25*#	6.72 ± 1.41	7.47 ± 10.04*	28.89 ± 20.22
1.0% Arginine-Bicarbonate	4.33 ± 0.17	5.17 ± 10.40*#	7.03 ± 1.09	0.95 ± 1.94*	30.87 ± 10.80
2.0% Arginine-Bicarbonate	4.37 ± 0.10	2.67 ± 6.53*#	7.18 ± 0.92	0.92 ± 2.25*	48.19 ± 30.22

*$P < 0.01$ versus water and sucrose groups; #$P < 0.01$ versus $NaHCO_3$ group. $AUC_{5.7}$ = area of plaque pH curve under 5.7; $AAC_{5.7}$ = area of plaque pH curve above 5.7; NA = not applicable

less time the plaque pH showed below 5.7 and smaller $AUC_{5.7}$ and larger $AAC_{5.7}$, the data were not statistically different among the arginine bicarbonate groups ($P > 0.05$, Table 1).

Acid buffer capacity of Arginine bicarbonate was higher than that of sodium bicarbonate

Although the 2% arginine bicarbonate contained bicarbonate equivalent to that of 85 mmol/L sodium bicarbonate, the acid buffer capacity of 2% arginine bicarbonate was 0.48, 1.78 times higher than that of 85 mmol/L sodium bicarbonate, which the acid buffer capacity was 0.27 ($P < 0.05$).

Discussion

In this study, we first used interdental plaque pH telemetry *in vivo* to show that 0.5 to 2% arginine bicarbonate rinse immediately and effectively neutralised the sucrose-induced decrease in dental plaque pH and maintained the plaque pH above the critical 5.7 until the end of the measurement. Without subsequent intervention, the plaque pH remained below the critical 5.7 for 83 min after the sucrose rinse, implying that the demineralisation of teeth would last for this long a time after consuming carbohydrates. A water rinse only reduced to some extent the depth of the plaque pH curve under pH 5.7, showing a minimal effect on the clearance of the acid in the dental plaque. Rinsing with sodium bicarbonate solution after the sucrose rinse also could not effectively neutralise the sucrose-induced decrease in plaque pH. These *in vivo* results indicated that the neutralising effect of arginine bicarbonate rinse could help inhibit the demineralisation of teeth after consuming carbohydrates.

The ability of arginine bicarbonate rinse to neutralise a sucrose-induced decrease in plaque pH was more likely due to the base formation of arginine rather than the acid buffer capacity of bicarbonate. Besides urea, arginine is another main nitrogen substrate for base formation in saliva and arginine supply is a practicable approach to enhance the base formation in plaque[1]. *In vitro,* arginine can be rapidly metabolised by oral bacteria to elicit a rise in environmental pH[11]. In the mouth, arginine is normally and mainly available from readily degradable peptides and less readily degradable proteins in saliva. The small arginine peptides are catabolised primarily by the arginine deiminase system (ADS), which yields ornithine, carbon dioxide and ammonia[12]. Because of the strong alkalinity of arginine, arginine bicarbonate was used as the arginine supplier in the *in vitro* study. It was found that arginine and small arginine peptides are essential for producing the pH-rise portion of the Stephan pH response; the sustained

pH rising effect was attributed to the metabolism of arginine of the solution[13,14]. Since arginine utilisation by mixed salivary bacteria occurs optimally at a pH of between 7 and 8 and is progressively less as the pH decreases to 5.0 and below, bicarbonate buffering can provide a more favourable pH for arginine degradation[12]. However, it was necessary to rule out whether or not the neutralising effect of arginine bicarbonate on sucrose-induced plaque pH was due to the effect of bicarbonate. Therefore, sodium bicarbonate solution was used as a control to compare its acid buffer capacity and effect on plaque pH with that of arginine bicarbonate. Although 85 mmol/L sodium bicarbonate solution contained bicarbonate equivalent to that of 2% arginine bicarbonate, rinsing with 85 mmol/L sodium bicarbonate solution after the sucrose rinse showed little effect on the sucrose-induced decrease in plaque pH, indicating that the neutralising effect of arginine bicarbonate on plaque pH was mainly due to the base formation of arginine, but due to the effect of bicarbonate. Although bicarbonate is considered the main buffer in saliva for neutralising plaque acid[15] and has been used in oral care products for caries prevention[16–18], our results showed that 85 mmol/L sodium bicarbonate alone appeared little effect on sucrose-induced plaque pH decrease, but induced instantaneous rapid pH rise, which was in accordance with the result of Imfeld[9].

Although the acid buffer capacity of arginine bicarbonate was larger than that of sodium bicarbonate and arginine bicarbonate rinse resulted in higher instantaneous pH rise, it appeared that the sustained pH rising effect in the plaque was still due to the metabolism of arginine, as the plaque pH dropped down soon after it reached its peak. Our *in vivo* results of interdental plaque pH telemetry confirmed the previous results of Abelson et al[19].

The important clinical potential of our results was that a low-concentration arginine bicarbonate rinse could be an effective method to prevent caries after carbohydrate consumption. Given that arginine bicarbonate was a long-time effective pH-rising agent, we strongly suggest that regular use of 0.5 to 2% arginine bicarbonate rinse after carbohydrate consumption could be a promising simple and effective anti-caries approach. This applies especially for high caries-risk people, such as patients receiving orthodontic treatment or dry mouth patients who cannot use chewing gum as a regular caries prevention measure. Moreover, arginine is a nutrient, and this makes the use of arginine bicarbonate rinse to be especially safe for children.

There could be a concern about whether the approach of arginine bicarbonate rinse is suitable for people with high caries-risk, since caries active individuals have lower ADS activity[5,6]. According to our results, 0.5 to 2% arginine bicarbonate should be effective even in high caries-risk people, because our subjects should belong to high caries-risk people. Certainly, the clinical effects of arginine bicarbonate in a large population of high caries-risk people should be examined.

Regular use of arginine bicarbonate may favour a base producing plaque microflora modification. A previous study showed that arginolytic acidogenic bacteria in the oral cavity can hydrolyse the endogenous arginine to produce the base and thus makes the acid-base pH response to glucose less acidic than that made by the urelytic acidogenic bacteria[11]. Moreover, continual presence of arginine favours the growth of arginolytic over nonarginolytic acidogens in healthy dental biofilms, thus it may help to prevent the emergence of a cariogenic mircroflora, maintaining a healthier, base producing plaque microflora[1,3]. Regular use of an arginine rinse can provide both the substrate and the means of increasing their utilisation by bringing about changes in plaque microbial composition. Future work is needed to clinically examine the effects of arginine bicarbonate rinse on caries prevention and the effect of long-term use of the rinse on concentration of base-producing bacteria.

The advantages of the telemetric method were previously documented[20,21]. In particular, the indwelling interproximal electrode system in this method was more sensitive than the plaque sampling and touch electrode system. These plaque-covered electrodes recorded the pH changes continuously, underneath an undisrupted plaque, at an interproximal site in which caries frequently occurs. Our results of the plaque pH response to the 10% sucrose rinse was consistent with the results of the previous studies[9,20]. The mean plaque pH value 10 min after the sucrose rinse was very similar among the groups, indicating that the consistency in the electrode response and rate and amount of interproximal plaque acid production were comparable and reproducible among the groups.

In conclusion, we showed that a subsequent arginine bicarbonate rinse could effectively neutralise a sucrose-induced prolonged decrease in plaque pH. Regular use of an arginine bicarbonate rinse after carbohydrate consumption could be an effective anti-caries approach.

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References

1. Kleinberg I. A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to Streptococcus mutans and the specific-plaque hypothesis. Crit Rev Oral Biol Med 2002;13:108–125.
2. Kleinberg I KJ, Chatterjee R, Craw D et al. Metabolism of nitrogen by the oral mixed bacteria. In: Kleinberg I ES, Mandel ID (ed). Saliva and Dental Caries. Washington, DC and London: Information Retrieval, 1979:357–377.
3. Kaufman N. Effect of an arginine dentifrice on arginolytic organisms in biofilm. J Dent Res 2005;84:3420.
4. Van Wuyckhuyse BC, Perinpanayagam HE, Bevacqua D et al. Association of free arginine and lysine concentrations in human parotid saliva with caries experience. J Dent Res 1995;74:686–690.
5. Nascimento MM, Gordan VV, Garvan CW et al. Correlations of oral bacterial arginine and urea catabolism with caries experience. Oral Microbiol Immunol 2009;24:89–95.
6. Gordan VV, Garvan CW, Ottenga ME et al. Could alkali production be considered an approach for caries control? Arch Oral Biol 2010;44:547–554.
7. Acevedo AM, Machado C, Rivera LE et al. The inhibitory effect of an arginine bicarbonate/calcium carbonate CaviStat-containing dentifrice on the development of dental caries in Venezuelan school children. J Clin Dent 2005;16:63–70.
8. Acevedo AM, Montero M, Rojas-Sanchez F et al. Clinical evaluation of the ability of CaviStat in a mint confection to inhibit the development of dental caries in children. J Clin Dent 2008;19:1–8.
9. Imfeld TN. Identification of low caries risk dietary components. Monogr Oral Sci 1983;11:1–198.
10. Pollard MA, Imfeld T, Higham SM et al. Acidogenic potential and total salivary carbohydrate content of expectorants following the consumption of some cereal-based foods and fruits. Caries Res 1996;30:132–137.
11. Wijeyeweera RL, Kleinberg I. Arginolytic and ureolytic activities of pure cultures of human oral bacteria and their effects on the pH response of salivary sediment and dental plaque in vitro. Arch Oral Biol 1989;34:43–53.
12. Kanapka JA, Kleinberg I. Catabolism of arginine by the mixed bacteria in human salivary sediment under conditions of low and high glucose concentration. Arch Oral Biol 1983;28:1007–1015.
13. Singer DL, Chatterjee R, Denepitiya L et al. A comparison of the acid-base metabolisms of pooled human dental plaque and salivary sediment. Arch Oral Biol 1983;28:29–35.
14. Wijeyeweera RL, Kleinberg I. Acid-base pH curves in vitro with mixtures of pure cultures of human oral microorganisms. Arch Oral Biol 1989;34:55–64.
15. Tavss EA, Eigen E. Factors affecting pH rise of suspended salivary sediment. Caries Res 1986;20:244–250.
16. Igarashi K, Lee IK, Schachtele CF. Effect of chewing gum containing sodium bicarbonate on human interproximal plaque pH. J Dent Res 1988;67:531–535.
17. Macpherson LM, Chen WY, Dawes C. Effects of salivary bicarbonate content and film velocity on pH changes in an artificial plaque containing Streptococcus oralis, after exposure to sucrose. J Dent Res 1991;70:1235–1238.
18. Nilner K, Vassilakos N, Birkhed D. Effect of a buffering sugar-free lozenge on intraoral pH and electrochemical action. Acta Odontol Scand 1991;49:267–272.
19. Abelson DC, Vratsanos SM, Mandel ID. Modification of dental plaque by arginine-urea to resist pH fall in vivo. Clin Prev Dent 1986;8:7–10.
20. Schachtele CF, Jensen ME. Comparison of methods for monitoring changes in the pH of human dental plaque. J Dent Res 1982;61:1117–1125.
21. Harper DS, Gray R, Lenke JW et al. Measurement of human plaque acidity: comparison of interdental touch and indwelling electrodes. Caries Research 1985;19:536–546.