Urea Rinse effectively Neutralises Sucrose-induced Decrease in Plaque pH

Xiao Ling WANG[1], Chuo Yue CHENG[1], Chun Ling GE[2], Bing WANG[2], Ye Hua GAN[3]

Objectives: *To investigate the neutralising effects of subsequent urea rinse on sucrose-induced decrease in plaque pH with interdental plaque pH telemetry.*
Method: *Six participants wearing partial lower prostheses which incorporated 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 by no subsequent rinsing or rinsing with either 15 ml of water, 0.25%, 0.50% or 1.00% urea solution, for 2 min. The plaque pH was continuously recorded for 120 min.*
Results: *Without subsequent rinsing, the plaque pH decreased at 10 min to 4.39 and stayed below the critical pH of 5.7 for 80 min following a sucrose rinse. Subsequent water rinse showed little effects on the sucrose-induced decrease in plaque pH, whereas subsequent urea rinses all immediately and effectively neutralised sucrose-induced decrease in plaque pH, and remained above the critical pH of 5.7 until the end of data collection.*
Conclusion: *Urea rinse could effectively counteract the pH fall following sucrose administration. These results strongly suggested that the regular use of low-concentration urea rinse after carbohydrate consumption may help prevent caries.*

Key words: *demineralisation, plaque pH, Stephan curve, sucrose, urea*

The integrity of the enamel depends on the acid-base balance within the dental plaque. Prolonged dental plaque acidification leads to demineralisation of the tooth. Caries is the result of long term acidity in the plaque due to oral bacteria fermenting dietary carbohydrate[1]. Reduction of acid production and removal of the acid from dental plaque is critical for caries prevention. Neutralisation of plaque acidification by alkali such as ammonia, after carbohydrate consumption, is thought to be an important method for caries inhibition. In recent years much attention has focused on the microbiology, ecology, biochemistry and physiology of alkali production in plaque[2-5]. Enhancing alkali production in plaque may be a promising strategy for caries control[5].

Urea is a major alkali-generation substrate in plaque and has a strong effect on the rise of plaque pH. As early as 1940 Stephan demonstrated that urea solution increases the pH in plaque[6]. However, later studies mainly focused on the exposure of plaque to urea solution before sucrose rinse and only observed an increase in the resting plaque pH, but there was little effect on reducing the depth and duration of a subsequent Stephan curve[7,8]. Recently, chewing urea-containing gum before exposure to sucrose also showed little effect on a subsequent Stephan curve[9]; but chewing urea-containing gum or rinsing with urea solution after a sucrose rinse accelerates the return of the plaque pH to a neutral pH[10,11]. Therefore, it is suggested that urea is likely to inhibit caries when it is used after consumption of fermentable carbohydrate, rather than before[9]. However, few studies in vivo were performed to investigate the

1 Department of Preventive Dentistry, Peking University School and Hospital of Stomatology, Beijing, P.R. China.
2 Department of Prosthodontics, Peking University School and Hospital of Stomatology, Beijing, P.R. China.
3 Central laboratory, Peking University School and Hospital of Stomatology, Beijing, P.R. China.

Corresponding author: Dr Ye Hua GAN, Central laboratory, Peking University School and Hospital of Stomatology, #22 Zhongguancun South Avenue, Haidian District, Beijing 100081, P.R. China. Tel: 86-10-82195518; Fax: 86-10-82193402. Email: kqyehuagan@bjmu.edu.cn
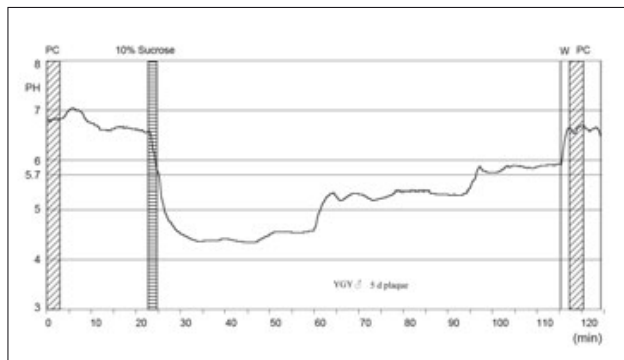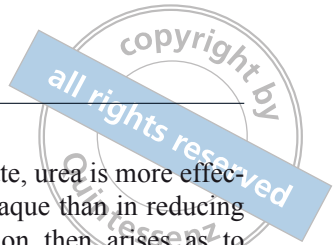
Fig 1 Telemetrically recorded interdental plaque pH profile of the subject YGY during and after sucrose rinse; W = water rinse, PC = paraffin chewing.
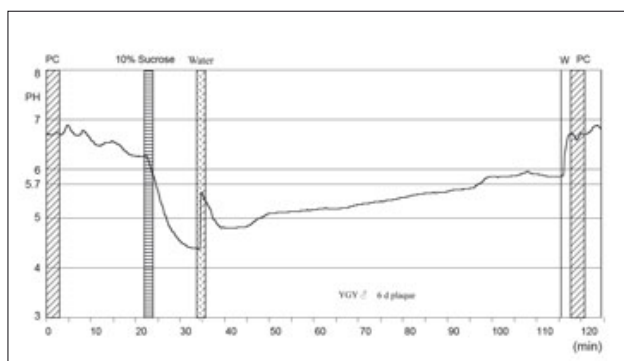

Fig 2 Telemetrically recorded interdental plaque pH profile of the same subject during and after sucrose rinse and subsequent water rinse; W = water rinse, PC = paraffin chewing.
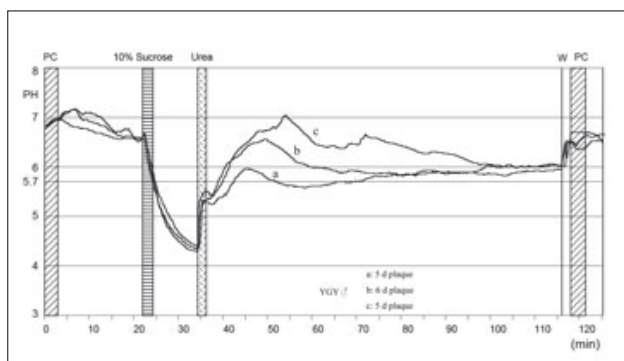

Fig 3 Telemetrically recorded interdental plaque pH profile of the same subject during and after sucrose rinse and subsequent 0.25% (a), 0.50% (b) and 1.00% (c) urea rinse; W = water rinse, PC = paraffin chewing.

neutralisation of plaque acidity by urea[10,11]. The caries preventive potential of urea rinse, especially with low concentrations, remains to be fully tested.

As an alkali generation substrate, urea is more effective in neutralising the acid in plaque than in reducing the acid production. The question then arises as to whether a low concentration of urea rinse after carbohydrate consumption can help prevent caries. The aim of this study was to examine the neutralising effects of low concentrations of urea rinse on sucrose-induced decrease in plaque pH using interdental plaque pH telemetry.

Material and methods

Subjects

Six healthy subjects (four males and two females) with a mean age of 63 years old (56 to 72 years old) were recruited; 2 or 3 missing teeth in the two premolar and molar regions of the mandible were used in the study. The subjects had between 7 to 13 (mean = 11) teeth of their own in the mandible and maxilla. All subjects had a stimulated salivary secretion rate > 0.60 ml/min, and the mean pH was 7.67. All the subjects had no unfilled cavities, periodontal disease or other oral diseases. Ethical approval for this study was obtained from the Ethics Committee of Peking University Health Science Center, 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[12]. Briefly, for each subject a mandibular partial prosthesis was fabricated incorporating a miniature glass pH electrode (W. Möller, Zurich, Switzerland). 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, Tokyo, Japan) and the original pH curve was scanned (Intuos3 VACOM, Saitama, Japan) and analysed by the computer software (TelDat, Version 1.5, Boling AG, Zurich, Switzerland). The electrode was calibrated with standard buffer pH 7 before each test session.

Plaque accumulation

The accumulation of plaque on the tip of the electrode was performed as described in detail previously[12]. The subjects were asked to wear the prostheses with clean electrodes remaining in place, not to remove the dental device or to alter their eating habits, and to refrain from all oral hygiene measures for the entire experi-

Table 1 Data (mean ± standard deviation) from plaque pH response curve to 10% sucrose rinse and subsequent water or urea rinse (n = 6).

Groups	pH at 10 min after sucrose rinse	Time for plaque pH to fall below pH 5.7	Highest pH after subsequent urea rinse	AUC 5.7 (arbitrary unit)	AAC 5.7 (arbitrary unit)
Sucrose	4.39 ± 0.17	80.00 ± 16.43		72.85 ± 19.56	
Water	4.28 ± 0.15	71.17 ± 18.45		32.84 ± 15.96**	
0.25% Urea	4.35 ± 0.12	9.33 ± 9.33*	6.00 ± 0.33	6.90 ± 5.85*	25.37 ± 21.72
0.50% Urea	4.25 ± 0.19	3.50 ± 4.14*	6.30 ± 0.77	2.14 ± 2.16*	47.84 ± 38.34***
1.00% Urea	4.35 ± 0.13	2.05 ± 4.28*	6.85 ± 0.75***	1.16 ± 1.73*	63.83 ± 32.12***

*$P < 0.01$ versus water and sucrose groups; **$P < 0.01$ versus sucrose group; ***$P < 0.05$ versus 0.25% urea group.
$AUC_{5.7}$ = area of plaque pH curve under 5.7; $AAC_{5.7}$ = area of plaque pH curve above 5.7.

mental period, except for when rinsing with water and to toothbrush without toothpaste. The pH measurement was carried out in the early morning either on the sixth or seventh day of plaque accumulation whereby participants had not eaten or drunk anything except water before the test.

Experiment procedure

The experiment procedure was performed as described previously[12,13], with minor modifications. The subjects rinsed with one of the solutions on each visit, with an interval of 1 week. In order to complete the experiment each subject attended five sessions in total. The experiment started with the chewing of paraffin for 3 min for plaque pH normalisation. After an initial period of 20 min, in order to establish a baseline value, the subjects rinsed with 15 ml of 10% sucrose solution for 2 min; 10 min after expectoration, the subjects rinsed with either 15 ml of tap water, 0.25%, 0.50%, or 1.00% urea 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 urea solutions were freshly prepared in distilled water before use. A total of 30 (6 × 5) telemetric curves were recorded. The pH values at 10 min after sucrose rinse, the time for plaque pH curve below 5.7, the area of plaque pH curve under 5.7 (AUC 5.7), the area of plaque pH curve above 5.7 (AAC 5.7), and the highest pH after urea rinse were calculated from the telemetric curves.

To test the stability of the urea solution, we also examined the effects of 0.5% urea solution stored at room temperature (RT) on plaque pH, at 6 months and 12 months, using the same procedure.

Statistical analysis

Statistical analysis was performed with SPSS 15.0 for Windows (SPSS, Chicago, USA). All data were presented as mean ± standard deviation. Two-way analysis of variance (ANOVA) was performed for comparison of differences between the groups. A value of $P < 0.05$ was considered statistically significant.

Results

Without subsequent treatment, the plaque pH decreased to 4.39 at 10 min after sucrose rinse and stayed below the critical pH of 5.7 for 80 min (Fig 1 and Table 1).

The plaque pH decreased to 4.24 at 10 min after sucrose rinse; after subsequent water rinse, the plaque pH jumped close to 5.7 and fell down quickly to half of the increased level and stayed below 5.7 for 71 min (Fig 2 and Table 1).

The pH of the three urea solutions was similar to that of water (data not shown). Ten minutes after sucrose rinse, the plaque pH decreased to a level similar to that of the water and control groups; after subsequent urea rinse, the plaque pH jumped close to 5.7, similar to rinsing with water, and quickly rose beyond the critical pH of 5.7, reaching the highest level within 20 min,

and then it gradually decreased, but remained above the critical pH of 5.7 until the end of the data collection (about 70 min), in all urea groups (Fig 3 and Table 1). The period of time in which plaque pH was below pH 5.7 and AUC 5.7 for urea groups were all significantly less than that of the water and control groups ($P < 0.01$). Our results indicate a regular trend; as concentration of urea rinse increased, the plaque pH showed less time below pH 5.7 and smaller AUC 5.7, although the data were not statistically different amongst the urea groups ($P > 0.05$, Table 1). The AAC 5.7 of the 0.50% and 1.00% urea groups was larger than that of the 0.25% urea group and the highest pH of the 1.00% group was higher than that of the 0.25% urea group ($P < 0.05$)

In addition, 0.5% urea stored at RT for 6 months and 12 months showed similar effects on plaque pH as that of freshly made urea (data not shown).

Discussion

In this study, we showed that even at concentrations as low as 0.25%, urea rinse could effectively neutralise sucrose-induced prolonged decrease in plaque pH. To the best of our knowledge, this is the first study to show the potential of low-concentration urea rinse for caries prevention. The plaque pH remained below the critical pH of 5.7 for up to 80 min after sucrose rinse. Water rinse showed little effect on this prolonged decrease in plaque pH. Therefore, the demineralisation of the tooth after carbohydrate consumption would last for a long period of time if without effective subsequent intervention. However, as low as 0.25%, urea rinse could immediately and effectively neutralise the sucrose-induced decrease in plaque pH, and maintain the plaque pH above the critical pH of 5.7 until the end of data collection (about 70 min). Considering that the demineralisation of the tooth beneath plaque occurs when the plaque pH is below 5.7, our data acquired in vivo with interdental plaque pH telemetry strongly suggested that the neutralising effects of low-concentration urea rinse could help inhibit demineralisation of the tooth after carbohydrate consumption and therefore prevent caries.

Plaque pH which remains below 5.7 for a long period of time is a key factor for caries development. After carbohydrate consumption, the acid formation in the plaque is more rapid than its removal from the plaque, resulting in a quick pH fall below the critical pH of 5.7 and therefore demineralisation of teeth. The more frequent the consumption of carbohydrate, the longer the period of time in which plaque pH stays below the critical pH of 5.7; this will lead to demineralisation of teeth beneath the plaque, overwhelming remineralisa-

tion and eventually the teeth will become decayed i.e. caries will be present. This is an important mechanism underlying caries development. Therefore, different agents and methods have been tested to interfere with this demineralising process to prevent caries. Based on our results, rinsing with urea, which has concentrations as low as 0.25% could immediately and effectively terminate the demineralising process.

Urea is a substrate for alkali generation and can be converted to ammonia and carbon dioxide by oral bacteria urease, with ammonia neutralising the hydrogen ion. Owing to the lower concentration of urea in saliva, the ammonia formation in the plaque can not counteract the acid formation after carbohydrate consumption. Therefore, after sucrose administration and without subsequent intervention, the return of the decreased plaque pH to above the critical pH of 5.7 was very slow and took 80 min according to our observations. However, subsequent urea rinse, as low as 0.25%, could effectively neutralise prolonged sucrose-induced decrease in plaque pH (Fig 3). This change of pH profile is associated with the production and clearance of the ammonia in the plaque, since the plaque ammonia concentration rises quickly to a maximum level within 10 min and then falls slowly after a urea rinse[14]. Our neutralising effects of urea rinse agreed with previous studies using urea-containing chewing gum or urea rinse after sucrose consumption[9-11]. Considering the neutralising effects of low-concentration urea rinse on the plaque pH, we strongly suggested that the regular use of low-concentration urea rinse after carbohydrate consumption may help prevent caries.

It was unexpected that the neutralising effects of a single urea rinse on the plaque pH lasted for such a long time, up to at least 70 min, with a concentration as low as 0.25%. Although the pH recovery effect of urea is also observed in a few studies of urea-containing chewing gum or urea rinse[9-11], their observation time is much shorter than ours. The prolonged neutralising effect of urea rinse was more likely due to the slower clearance of ammonia in the plaque, rather than the continuous conversion of residual urea into ammonia in the plaque or saliva by bacteria urease, since the urea level in the plaque and saliva returns to baseline within 20 min after urea rinse, whilst ammonia levels in the plaque do not return to baseline until at least 30 min have passed[14]. The prolonged neutralising effect of urea rinse is particularly favourable for preventing caries. In addition, the rapid penetration and breakdown of the urea in the interdental plaque, which is not easily removed by regular toothbrushing, is also more beneficial for caries prevention. The variation of our data was large, which

might be related to individual variations in bacteria for consuming urea, saliva composition, flow rate, pH etc.

The important clinical potential of our results was that low-concentration urea rinse could be an effective method to prevent caries after carbohydrate consumption. Given that urea was effective as a long-term agent in causing increases in pH, we strongly suggested that the regular use of 0.25% to 1.00% urea rinse after carbohydrate consumption could be a simple and effective anti-caries approach, especially for high-caries risk individuals, such as patients receiving orthodontic treatment or dry mouth patients who could not use chewing gum as a regular caries prevention measure. These individuals could use 0.5% to 1.0% urea rinse, in order to ensure slightly stronger neutralising effects. Even 1.0% urea solution shows no taste[9,15]. After storage at room temperature for 1 year its effects on plaque pH showed no difference from freshly made urea solution. This means that the storage of urea rinse is stable and convenient. From an overall oral health point of view and our results, we suggested 0.25% to 1.00% urea for caries prevention after carbohydrate consumption. In addition, urea is a product of human metabolism and exists in saliva. Application of low concentrations of urea solution as mouth rinse should be safe, despite the fact that the majority of it will be expectorated. These features ensured the use of urea rinse was clinically practicable for preventing caries, especially for those who can not use chewing gum as a prevention measure. Future studies are needed to clinically examine the effects of low-concentration urea rinse on caries prevention and the effect of the long-term use of urea rinse on the concentration of base-producing bacteria, and also the alkali generating potential in plaque.

The anti-caries effect of urea was already clinically proven in chronic renal failure patients, showing that the patients have significantly higher salivary urea and a lower prevalence of caries[16]. This convincing clinical study looking at the anti-caries effects of urea was performed about 30 years ago. We are wondering why urea has not been widely used for caries prevention, although its neutralising effects were discovered since the 1940s. It is possible calculus formation after urea use is of chief concern. Clinical studies demonstrate contradictory results between urea application and calculus formation[15,17]. The discrepancy could be due to different testing conditions, including whether or not the solution or gum contains extra ions. Future studies are still needed to test the influence of low-concentration urea rinse on calculus formation.

There could be issues in terms of the approach of urea rinse, which is suitable for people with high-caries risk, since individuals with caries have lower urease activity[3,4]. According to our results, it was already demonstrated that urea rinse was still effective in people with high-caries risk, because our subjects had only 7 to 13 teeth of their own and should belong to the high-caries risk population. It is important that future studies should examine the clinical effects of low-concentration urea rinse in a large population with high-caries risk.

In this study, the interdental plaque pH response was measured continuously with the telemetric method for 120 min. The advantages of the telemetric method were previously documented[16,18]. In general, the telemetric method is a sensitive and reliable method to analyse the biochemical activity of the plaque on the interproximal site, which is caries susceptible[16]. Therefore, the data acquired with this method would help in understanding why plaque pH continuously changes after sucrose consumption. This method is a useful tool to evaluate the effects of caries-preventive agents.

Our plaque pH response to 10% sucrose rinse was consistent with the results of others[12,18]. The mean plaque pH value 10 min after sucrose rinse was very similar amongst the groups, indicating that the consistency in the electrode response and the rate and amount of interproximal plaque acid production, were comparable and reproducible amongst the groups.

In conclusion, we showed that subsequent urea rinse could effectively neutralise prolonged sucrose-induced decrease in plaque pH. The regular use of urea rinse after carbohydrate consumption might help caries prevention.

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