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Short Communication

Evaluation of the synergistic antibacterial effect of chlorhexidine solution 0.2% and phosphoric acid gel 37% against *Streptococcus mutans* during fissure sealant therapy: An in vitro study

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Abstract

Background: Pit and fissure sealant therapy is widely used to prevent caries of the occlusal surfaces of posterior teeth. In this treatment, failure is not uncommon. *Streptococcus mutans* is the most important bacterium that initiates and progresses caries lesions. This study aimed to evaluate the synergizing antibacterial effect of chlorhexidine solution 0.2% and phosphoric acid gel 37% against *S. mutans* during fissure sealant therapy to reduce the risk of failure. The disc diffusion method was used to evaluate the effects of the materials. This method has been applied in many studies.

Methods: The antibacterial effect of the two materials was evaluated using the disc diffusion method. *S. mutans* was acquired from the Iranian Industrial Bacteria and Fungi Collection Center (code ATCC35668) and was cultured in a laboratory environment on three plates containing a blood agar culture medium. In each plate were placed four discs: a disc that had been soaked in 0.2% chlorhexidine solution, a disc that had been soaked in 37% phosphoric acid, a disc that had been soaked in sterile distilled water as a negative control, and a disc containing ciprofloxacin antibiotic as a positive control. The plates were placed in the incubator for specific periods. The diameter of the bacteria inhibition halo around the test and control discs was measured and compared with each other. SPSS 26 statistical software was used for data analysis. The Mann-Whitney test was performed, and descriptive statistics were used to describe the halo of the bacterial inhibition growth.

Results: Phosphoric acid gel 37% showed significant anti-*S. mutans* effect. The antibacterial effect of chlorhexidine 0.2% solution was also comparable to that of the positive control disc.

Conclusion: Applying chlorhexidine solution on the occlusal surfaces shortly before the sealant therapy can reduce the risk of decay under the sealants.

Keywords: Chlorhexidine, Phosphoric acid, Pit and fissure sealants, *Streptococcus mutans*

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Introduction

Pit and fissure sealant therapy is widely used to prevent caries of the occlusal surfaces of posterior teeth. The effectiveness of fissure sealant therapy has been confirmed in many studies.[1-4] Although it is assumed that pit and fissure sealants can prevent caries in occlusal surfaces, they are currently applied to control and manage lesions with primary caries in occlusal and proximal surfaces.[5] The sealant forms a physical barrier between the oral environment and the fissures of the teeth, which are the main places for early caries.[6] The result of sealant therapy depends on the amount of retention and microleakage of the sealant in the teeth.[7,8] One of the causes of microleakage is contamination by bacteria. Microleakage around sealants allows bacteria to penetrate and cause secondary caries. The progress of these carious lesions causes pulp exposure in children.[9] Deep pits and fissures cause almost 90% of cavities in permanent posterior teeth. Although the acid etch agent removes microorganisms from the surface of the enamel,[10] in teeth with primary caries lesions and deep pits and fissures, some bacteria remain deep in the pits and fissures and under the sealant.

In some cases, the bacteria slowly spread to the dentin. *Streptococcus mutans* is the most important cause of the initiation and progression of caries lesions. The current study evaluated the antibacterial effect of 37% phosphoric acid and 0.2% chlorhexidine by disc diffusion. This method has been applied in many studies.[11] Pits and fissures are eight times more susceptible to decay than smooth surfaces.[12] Therefore, fissure sealant therapy in caries-prone teeth can be a physical barrier to microorganisms and food particles. However, obtaining complete isolation in young children during dental procedures is difficult. The placement of sealants is a very sensitive technique and is affected by several factors, including patient cooperation and environmental contamination.[13] Recurrent caries lesions are of two types: (*a*) external lesions caused by plaque accumulation and (*b*) internal lesions caused by microleakage between the tooth and the restoration. Microleakage is created around the sealants, creating a path for the penetration of oral fluids and microorganisms into the fissures and causing secondary caries.[14] Using an antibacterial agent reduces the accumulation of microbial plaque on tooth surfaces, thereby eliminating secondary caries.[15]

No study has investigated the synergistic effect of chlorhexidine and phosphoric acid in reducing *S. mutans* infection. Therefore, this study evaluated the synergizing antibacterial effect of chlorhexidine solution 0.2% and phosphoric acid gel 37% against *S. mutans* during fissure sealant therapy to reduce secondary caries.

Methods

Streptococcus mutans was acquired from the Iranian Industrial Bacteria and Fungi Collection Center (code ATCC35668). After acquisition, the bacteria were kept in tryptone soy broth (TSB) at -70 °C. A concentration of half McFarland *S. mutans* was prepared (because half McFarland standard turbidity was required). After preparing the half McFarland *S. mutans* concentration, it was cultured as a spread on the three plates containing blood agar culture medium by sterile swap. Then, in each plate were placed four discs, each with a diameter of 5 mm: a disc that had been soaked in 0.2% chlorhexidine solution (Irannajo Co., Iran), a disc that had been soaked in 37% phosphoric acid gel (NikDarman Co., Iran), a disc that had been soaked in sterile distilled water as a negative control, and a disc containing ciprofloxacin antibiotic (Sanamed Co., Iran) as a positive control. Then, the plates containing the discs were placed in candle jars to supply 10% of carbon dioxide and in an incubator with a temperature of 37 degrees Celsius for 24–48 hours. After specific periods, the diameters of the bacteria inhibition growth halo around the test and control discs were checked, measured, and compared with each other. The results were presented in tables and graphs. SPSS 26 statistical software was used for data analysis. Descriptive statistics, including mean and standard deviation, were used to describe the bacterial inhibition halo. The Mann-Whitney test was performed to compare the diameter of the halo in different materials after 24 hours, 48 hours, 72 hours, and one week.

Results

The results indicated that the largest bacterial inhibition halo in the first 24 hours was related to the positive control (ciprofloxacin). In the case of the negative control (distilled water), no inhibition halo was formed over time. Concerning chlorhexidine and phosphoric acid, in the first 24 hours, the diameter of the inhibition halo of phosphoric acid was slightly (18.0 ± 0.1 mm) larger than that of chlorhexidine (17.7 ± 2.5 mm). After 48 hours, the diameter of the inhibition halo for both phosphoric acid and chlorhexidine increased. Then, the inhibition halo did not change over time (72 hours and 168 hours) for these two materials (Table 1).

After 24 hours, according to the Mann-Whitney test, ciprofloxacin had a significantly larger inhibition halo than phosphoric acid ($P=0.05$). This difference was not significant compared to chlorhexidine ($P=0.08$). The difference between phosphoric acid and chlorhexidine was insignificant ($P=0.1$). Pairwise comparisons based on the Mann-Whitney test at time intervals of 48 hours, 72 hours, and one week showed that phosphoric acid had a significantly larger inhibition growth halo than chlorhexidine ($P=0.05$). Ciprofloxacin was not significantly different from phosphoric acid ($P=0.08$) and chlorhexidine ($P=0.08$) (Table 2).

Discussion

The literature shows that a 37% phosphoric acid solution can reduce bacteria in the fissures and pits of the occlusal surfaces of the teeth when used for etching and sealant

Table 1. The mean diameter of the inhibition growth halo around the different materials at different points in time

Material	Time (h)	Mean (SD) (mm)	Minimum (mm)	Maximum (mm)
Distilled water	24, 48, 72, 168	0.0 (0.0)	0.0	0.0
Phosphoric acid	24	18.0 (1.0)	17.0	19.0
Phosphoric acid	48	23.0 (1.0)	22.0	24.0
Phosphoric acid	72	23.0 (1.0)	22.0	24.0
Phosphoric acid	168	23.0 (1.0)	22.0	24.0
Chlorhexidine	24	17.0 (2.5)	15.0	20.0
Chlorhexidine	48	18.0 (1.5)	17.0	20.0
Chlorhexidine	72	18.0 (1.5)	17.0	20.0
Chlorhexidine	168	18.0 (1.5)	17.0	20.0
Ciprofloxacin	24	21.0 (1.5)	20.0	22.0
Ciprofloxacin	48	21.0 (1.0)	20.0	22.0
Ciprofloxacin	72	21.0 (1.0)	20.0	22.0
Ciprofloxacin	168	21.0 (1.0)	20.0	22.0

Table 2. Comparisons between the mean diameter of the inhibition growth halo around the different materials at different points in time

Time (h)	Material		*P* value *
24	Phosphoric acid	Chlorhexidine	0.1
		Ciprofloxacin	0.05
	Chlorhexidine	Ciprofloxacin	0.08
48	Phosphoric acid	Chlorhexidine	0.05
		Ciprofloxacin	0.08
	Chlorhexidine	Ciprofloxacin	0.08
72	Phosphoric acid	Chlorhexidine	0.05
		Ciprofloxacin	0.08
	Chlorhexidine	Ciprofloxacin	0.08
168	Phosphoric acid	Chlorhexidine	0.05
		Ciprofloxacin	0.08
	Chlorhexidine	Ciprofloxacin	0.08

*Mann-Whitney test.

therapy.[16] The present study confirmed the results of previous studies regarding the antibacterial effect of 37% phosphoric acid. On the other hand, some studies and reports indicate the occurrence of caries under the sealant material inside the pit and fissures of the occlusal surfaces of the teeth.[16]

The antibacterial activity of chlorhexidine and its ability to eliminate a wide range of gram-positive and -negative bacteria make it applicable as a widely used mouthwash to help treat oral infections and ulcers. Various studies have determined this effect. The studies of Ribeiro et al,[17] Shah et al,[18] and Mensitieri et al[19] showed that chlorhexidine mouthwash is effective in reducing *S. mutans*. Since 1980, adding chlorhexidine in various forms and percentages to glass ionomer cement and composite resins has been tested to provide antibacterial activity. Shanmugaavel et al showed that adding 1% chlorhexidine to glass ionomer and resin sealants causes antibacterial activity against *S. mutans* and *Lactobacillus* in both sealants for up to 30 days.[15] De Castilho et al. investigated the effects of adding 1.25% chlorhexidine in in-vitro and in-vivo experiments.[20] The effects of adding 1.25% chlorhexidine to resin-modified glass ionomer cement (RMGIC) in an in-vitro study led to improved antibacterial properties. The in-vivo results also showed the eradication of *S. mutans* in the restored cavities with resin-modified glass ionomer cement RMGIC after 3 months.[15]

However, no study has been done on preparing the occlusal surface with chlorhexidine before applying 37% phosphoric acid gel and its effect on *S. mutans*. The results of this study showed a positive effect of chlorhexidine. The findings demonstrated the antibacterial effect of chlorhexidine solution before applying phosphoric acid.

If phosphoric acid gel and chlorhexidine solution are mixed, the antibacterial effect of their mixture may be stronger than that of each of them. For example, if an empty antibiogram disc is dipped in chlorhexidine solution and then in phosphoric acid gel, the bacterial inhibition halo in the *S. mutans* culture medium may be larger. However, phosphoric acid may have a negative effect on chlorhexidine and reduce its antibacterial properties, or chlorhexidine may change the pH of phosphoric acid and negatively affect its etching properties.

Limitations and suggestions

The current study's sample size was small, and the anti-*S. mutans* effects of the two materials were evaluated for a limited time. Therefore, it is suggested that a study with a larger sample size and longer time be conducted. Also, we suggest another study to evaluate the anti-*S. mutans* effect of a mixture of chlorhexidine 0.2% and phosphoric acid 37%.

Conclusion

Based on this laboratory study, 37% phosphoric acid gel showed a significant antibacterial effect. The antibacterial effect of 0.2% chlorhexidine solution was also comparable to ciprofloxacin. Although the antibacterial effect of chlorhexidine solution was less than that of phosphoric acid gel, this study indicates that applying chlorhexidine solution on the occlusal surfaces shortly before the sealant therapy process can further reduce the number of bacteria. This can reduce the risk of decay under the sealant materials.

Authors' Contribution

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Competing Interests

The authors have declared that no conflict of interest exists.

Ethical Approval

This project was approved by the Kerman University of Medical Sciences Ethics Committee (code of ethics IR.KMU.REC.1400.047).

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