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Effect of cheese and casein phosphopeptide-amorphous calcium phosphate on erosive lesions of primary teeth enamel following exposure to amoxicillin and ibuprofen syrups: An *in vitro* study

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ABSTRACT

Background: The acidic component of liquid medicinal syrups used by pediatric patients may cause erosion and partial demineralization. This study aimed to evaluate the effect of cheese and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on erosive lesions of primary teeth enamel following exposure to amoxicillin and ibuprofen syrups.

Materials and Methods: In this *in vitro* study, 60 noncarious deciduous molars were used. After measuring the surface microhardness of the samples, they were randomly separated into two groups and immersed in either amoxicillin or ibuprofen for 1 min three times per day. CPP-ACP, cheese, and artificial saliva were then applied to each of the three subgroups (n = 10). After each immersion time, 10 min of therapy was given. Between treatment intervals, the samples were kept in artificial saliva. The microhardness was remeasured after 1 week. Data were analyzed using SPSS software through repeated-measures ANOVA (α = 0.05).

Results: All samples' microhardness reduced considerably after immersion in liquid pharmaceuticals (amoxicillin [84.9 kgf/mm^2] and ibuprofen [75.1 kgf/mm^2]), but increased significantly following exposure to therapeutic solutions. There was no difference between the amoxicillin-cheese and amoxicillin-CPP-ACP subgroups (P = 0.975). A statistically insignificant difference was found between the ibuprofen group and the ibuprofen-CPP-ACP subgroup (P = 0.499).

Conclusion: As a result, cheese and CPP-ACP can be utilized to remineralize erosive lesions caused by amoxicillin or ibuprofen exposure.

Key Words: Amoxycillin, casein phosphopeptide-amorphous calcium phosphate, cheese, demineralization, ibuprofen, remineralization

INTRODUCTION

Dental erosion occurs when nonbacterial acids dissolve the enamel and dentine.[1] Acidic drinks such as soda, sports drinks, and fruit juices are becoming more widely available and used, raising the prevalence of this condition. Acids are typically derived through food, pharmaceuticals, occupational exposure, and lifestyle activities.[2-4]

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Pediatric liquid medications such as amoxicillin and ibuprofen are widely used and commonly accepted by children and parents; however, they can increase the risk of dental erosions. Acidic formulations are assumed to improve chemical stability, physiological compatibility, and flavor.[5,6] Along with acidic substances, a high frequency and/or prolonged consumption (≥2 times per day), having meals and snacks before bedtime, highly viscous saliva, and reduced flow rate contribute to an increased risk of erosion.[5,7]

Dental erosion prevention and reversal strategies have been developed. Fluoride effectively prevents acids from demineralizing the teeth. However, some concerns exist regarding the side effects of its extensive use on the body organs in normal individuals and dental fluorosis. In addition, each fluorapatite molecule needs calcium and phosphate to form. Consequently, there must be an effective substitute for fluoride in remineralizing the early stages of tooth decay.[8]

Tooth decay and teeth remineralization can be prevented by consuming calcium- and phosphate-rich foods, such as milk and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[9] Remineralizing might be enhanced through milk consumption or eating cheese. Their higher calcium and phosphate levels (compared to water or saliva) promote them to be great donors of calcium and phosphate required for remineralization. Although milk and cheese consumption is frequently recommended to help re-harden demineralized dental hard tissues, a few research have investigated their effects on enamel remineralization.[10]

The calcium and phosphate ion gradients and ion pairs inside a subsurface lesion can be preserved using CPP-ACP nanoclusters. Hydroxyapatite or fluorapatite, which works as an inhibitor of enamel demineralization and promotes remineralization, is formed in the lesion fluid because of the greater ion concentration.[11] Antibiotic syrups are inevitable in specific conditions despite their associated risk of dental erosion. Yet, calcium phosphorus-containing agents can be a promising option to compensate for the negative effects. This study aimed to assess the effect of cheese and CPP-ACP on erosive lesions of primary teeth enamel following exposure to amoxicillin and ibuprofen syrups.

MATERIALS AND METHODS

The Shahed University Ethics Committee approved this *in vitro* study (IR.SHAHED.REC.1399.012). The teeth were collected from the Department of Pedodontics, Shahed Dental College, Tehran, Iran. Teeth with caries and not having intact enamel surfaces were excluded from this study. Finally, 60 human primary molars were used for this *in vitro* study, which was carried out using previously extracted teeth preserved in Tehran tap water at 25°C until needed. Before the operation, the teeth were scaled by the hand and cleaned using a fine-grain pumice-water slurry (Dental AG Ltda, Sa˜o Paulo, SP, Brazil) and Robinson bristle brushes (Labordental Ltda, Sa˜o Paulo, SP, Brazil) in the low-speed handpiece for 30 s. Using a micromotor handpiece, each tooth was cut in longitudinal sections with a diamond disc. The teeth were mounted in a 30 mm × 20 mm mold containing self-cure acrylic (Acropars Re, Marlic Medical Industries Company, Tehran, Iran). The external enamel buccal surface was polished using abrasive papers (800-1500-2500 grit) (Matador Wasserfest, Germany) and water for <60 s.

After drying, a Vickers microhardness tester (Shimadzu M-g5037; Shimadzu Corporation, Kyoto, Japan) with a 50 g load applied for 10 s on 3 points was used to measure the baseline surface microhardness. The samples were separated into two experimental groups (n = 30) and immersed in either amoxicillin (250 mg/mL; pH: 5.68, Cosar Pharmaceutical Company, Tehran, Iran) or ibuprofen syrup (120 mg/mL; pH: 4.42, Alborz Darou, Tehran, Iran) for 1 min three times a day for 1 week to develop demineralized lesions.[12] ACP-CPP (Tooth Mousse, GC, Tokyo, Japan), cheese (pH: 5.9, Pegah, Karaj, Iran), and artificial saliva (KIN Hidrat: AQUA, PEG-40 hydrogenated Castrol oil, xylitol, sodium saccharin, sodium methylparaben, potassium chloride, potassium phosphate, menthol, citric acid, aroma, ethylparaben, calcium chloride, sodium chloride, 2-bromo-2 nitropropane-1,3-diol, sodium propylparaben, potassium thiocyanate, and magnesium chloride) (Kin Hidrate, Kin, Spain) were then applied to each of the three subgroups (n = 10). The cheese had been softened to create a paste that could adhere to the tooth surfaces. Once the cheese was in the appropriate form, it was applied to the enamel surface of the prepared tooth samples. The application was conducted by immersing the tooth in the cheese mixture. After the application of cheese, the samples were likely rinsed with water to remove any excess. After each immersion time, 10 min of therapy was given. During the time between treatments, the samples were stored

in artificial saliva. After 1 week, the microhardness of the surface was remeasured. All procedures would have been conducted under controlled conditions to ensure consistency and reliability of results.

The data were analyzed using SPSS software (version 24, SPSS Inc., Chicago, IL, USA). ANOVA with repeated measurements analyzed the data. The level of significance was set at $P < 0.05$.

RESULTS

The mean surface microhardness of the two groups did not differ significantly at the beginning of the experiment. The mean surface microhardness decreased following exposure to amoxicillin (84.9 kgf/mm^2) and ibuprofen syrups (75.1 kgf/mm^2). Surface microhardness was increased significantly following exposure to therapeutic solutions. Surface microhardness was not significantly different between the amoxicillin-cheese (118.6 kgf/mm^2) and amoxicillin-CPP-ACP subgroups (120.7 kgf/mm^2) after applying the remineralizing agents ($P = 0.975$). A statistically insignificant difference was found between the ibuprofen-cheese group and the ibuprofen-CPP-ACP subgroup [$P = 0.499$, Table 1].

DISCUSSION

Despite several studies on dental erosion, the erosive impact of pediatric liquid medications on primary teeth is still unknown. Due to the acid in their composition, some liquid drugs may be highly erosive.[12] Calcium- and phosphate-containing compounds are among the substances that can increase the hardness and strength of tooth enamel after erosion. Such compounds added to various pharmaceutical and food products are claimed to contribute to tooth remineralization.[13]

This study revealed that exposing the teeth to amoxicillin and ibuprofen syrups decreased enamel microhardness. Liquid medicinal syrups can decrease the pH of dental plaques and, consequently, the enamel microhardness.[14] Many similar studies documented that medicinal syrups decreased the enamel microhardness, which was in line with the present findings.[6,7,15-17] Mahmoud and Omar[12] found that pediatric liquid medicines such as amoxicillin and ibuprofen syrups significantly affected the erosion in primary tooth enamel due to their pH, sugar content, and calcium dissolution ability. However, they treated the samples for 1 min three times a day for 14 and 28 days. The present study noted microhardness decrease within 7 days, the commonly prescribed duration for syrup administration in pediatric patients.

Cheese and CPP-ACP were also discovered to be able to remineralize erosive lesions induced by amoxicillin and ibuprofen exposure in this investigation. ACP nanoclusters may be formed through calcium and phosphate ions binding by CPP. As a result, the CPP-ACP nanoclusters can maintain the subsurface lesion's concentration gradients and ion pairs. Hydroxyapatite or fluorapatite crystals are formed as the ion concentration in the fluid lesion rises, preventing enamel demineralization and promoting remineralization.[11] Fluoride and calcium ions can be maintained at the tooth surface by CPP-ACP binding to the plaque and pellicle of the teeth. A pool of calcium and phosphate is formed when CPP is combined with ACP, helping to keep saliva saturated. Because it increases calcium and phosphate levels in the plaque's subsurface lesions, CPP-ACP may help keep plaque calcium and phosphate levels high.[18]

Because of its high casein and whey protein content, cheese has long been regarded as a preventive diet that provides other advantages beyond remineralization. The natural CPP and higher calcium levels in the plaque maintain the enamel, minimize demineralization, and promote remineralization. Saliva stimulation is another factor contributing to cheese's remineralization ability. Chewing cheese increases saliva production, which helps neutralize acids and provides additional calcium and phosphate ions for remineralization. Saliva also contains various proteins and enzymes that aid remineralization.[19] Higher calcium levels in plaque are also associated with cheese consumption. Calcium is essential for remineralization, as it helps form hydroxyapatite crystals, the primary mineral component of tooth enamel.[20,21] When cheese is consumed, it increases the availability of calcium ions in the oral environment,

Table 1: The mean microhardness of groups and subgroups before and after treatment with remineralizing agents

Groups	Before treatment (kgf/mm^2)	After treatment (kgf/mm^2)	*P*
Amoxicillin + ACP-CPP	347.2	120.7	0.975
Amoxicillin + cheese	338.3	118.6	
Ibuprofen + ACP-CPP	354.2	105.3	0.499
Ibuprofen + cheese	318.0	120.7	

ACP-CPP: Amorphous calcium phosphate-casein phosphopeptide

promoting the remineralization of enamel. Cheese is generally included in a normal daily diet; therefore, it can be a more convenient option than synthetic agents to replenish the demineralized surface with calcium and phosphate.[22] CPPs are derived from casein, a milk protein found in cheese. However, it is important to note that the remineralization mechanisms of cheese and CPP-ACP may differ slightly, as CPP-ACP is a synthetic agent specifically designed to promote remineralization, while cheese is a natural food product with multiple components contributing to its remineralization ability.

In a comparative study, Grewal *et al*.[22] discovered that cheese and CPP-ACP-based synthetic compounds could potentially remineralize enamel effectively. Grewal *et al*. evaluated the remineralization effect of these two agents on erosive lesions *in situ*. In contrast, the current study assessed the remineralization effect of these two agents on erosive lesions caused by exposure to liquid drugs, specifically amoxicillin and ibuprofen syrups. Following the findings of this investigation, Maden *et al*.[23] saw that CPP-ACP can reverse enamel degradation caused by carbonated soft drink consumption in primary teeth. In that investigation, a soft drink was used as the erosive agent, and the enamel's surface roughness was determined using profilometry.

Rezvani *et al*.[24] and Haghgou *et al*.[25] reported that the mean enamel microhardness increased after exposure to CPP-ACP, which was consistent with the present findings. However, Rezvani *et al*. evaluated beverages' effect on permanent teeth and compared the effects of whey extract and CPP-ACP. In contrast, Haghgou *et al*. induced erosive lesions by immersing permanent teeth in beverages and compared these effects to tricalcium phosphate and CPP-ACP.

Thierens *et al*.[11] discovered that long-term use of CPP-ACP and CPP-ACP fluoride in conjunction with a conventional toothpaste was found to have remineralizing effects on subsurface carious lesions recovered *in vitro*. It was analogous to the current study in that it examined the effect of CPP-ACP on permanent teeth and the remineralization of subsurface caries lesions. This study's exposure time (1 min/3 time day for 7 days) was the most often used regimen for amoxicillin 250 mg/mL syrup.

CPP-ACP can be added to various food deliveries, such as gum, candy, ice cream, and other dairy products.[26,27] Several studies and clinical trials have approved CPP-ACP products' remineralizing potential and success rate with or without fluoride. However, as documented in the current study, very few have investigated the efficacy of natural foods containing calcium-phosphate in replenishing the lost calcium/phosphate ions from enamel. It is important to note that while the study provides valuable insights into the effects of certain substances on tooth enamel *in vitro*, actual conditions in the oral cavity, including saliva flow, oral hygiene habits, and individual patient behaviors, can significantly influence outcomes. Therefore, recommendations for patients must consider a comprehensive approach to oral health care and not solely rely on the results from *in vitro* studies. More research is needed to determine the effect of analgesics on primary tooth enamel microhardness and investigate additional remineralizing agents in the oral environment.

CONCLUSION

Most medicines' syrups used in children, including antibiotics, have acidic pH to increase their shelf life or effectiveness. This causes them to erode the tooth surface in repeated use. According to the present findings, cheese and CPP-ACP can be used as remineralizing agents for the erosive lesion induced by amoxicillin and ibuprofen.

Data availability

The data used to support the findings of this study are available from the corresponding author on request.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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