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Assessment of Protective Effect of Amorphous Calcium Phosphate-Caseine Phosphopeptide and Silicone Oil against the Erosive Property of Iron Drop: An in-Vitro Investigation in Primary Teeth

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Abstract

Background: One of the complications of Iron drop recommended for 6-24 months children is the potential reduction in microhardness of primary tooth enamel because of low pH. The objective of this study is to assess the protective effect of amorphous calcium phosphate caseine phosphopeptide (ACP-CPP) and silicone oil in primary teeth.

Materials and Methods: Thirty extracted primary anterior teeth were divided into three equal groups. The initial micro hardness was measured by Vicker's microhardness tester. The first group without a protective layer and the second and third group after application of ACP-CPP and silicone oil respectively, were immersed in iron drop. Microhardness was remeasured. One tooth in each group along with a tooth not exposed to iron drop were randomly chosen for SEM qualitative analysis. Analysis was performed with Repeated measures ANOVA with SPSS-18.

Results: All groups exhibited significant decrease of micro hardness (p=0.001), however, no contrasting pattern was found between various groups.

Conclusion: Neither ACP-CPP nor silicone oil could not provide a significant protection against micro hardness reduction after exposure to iron drop

Introduction

Iron, provides cells with a constant supply of oxygen as its primary role in the body. It also functions as a cofactor for many enzymatic reactions and is essential to the immune system [1-3]. American Academy of Pediatrics (AAP) recommends a prophylactic dose of oral iron (1 mg/kg per day) for exclusively breast-fed infants after 4 months of age [4]. In Iran, iron drops are routinely prescribed by pediatricians who are started at the age of 6 months. Tooth staining is one of the most prevalent reasons of incompliance pointed by parents. There is also a general belief in public that iron drops cause caries and excessive staining. Unfortunately, this belief offers some parents an excuse to disregard the regular consumption [5, 6]. Any medication with low pH and high titrable acidity, however, coming in direct contact with tooth surface has erosive potential [7]. Erosion, loss of dental hard tissue by acids of non-bacterial origin may roughen the surface that predisposes the tooth to stain deposits, plaque formation with further tooth decay. Parents may mistake these dark stains as tooth decay [8]. In preventive dentistry, Amorphous Calcium Phosphate Casein Phosphor Peptide (ACP-CPP) is one of the most common materials suggested to protect teeth from demineralization and has shown a therapeutic effect against enamel defects such as white spots, hypersensitivity, and also erosion [9]. Moreover, silicone oil has been introduced that can act as a resistant layer on tooth avoiding direct contact between Iron and tooth surface, hence preventing from erosion [10]. We conducted this in vitro study to evaluate the erosive effect of iron drop on primary tooth enamel, and to evaluate the protective effect of silicone oil and ACP-CPP towards erosion.

Materials and Methods

This in vitro study was conducted on 30 human anterior primary teeth (A, B, C). The samples were collected from public dental clinics. Included teeth were extracted not more than 3 months ago, without caries and white spot present on tooth surface and had no macroscopic signs of wear. The teeth were stored in normal saline 0.9%. The storage media was changed daily during the first week and then weekly until the beginning of the experiment for 10 consecutive weeks. Sterilization was not done to avoid the possible deleterious effect of heat and chemicals on microhardness. The apex of teeth was sealed with sticky wax and was mounted on microscope slides with a two component adhesive (mitreapeal cyanoacrylate adhesive, Turkey). The teeth were situated with their facial surface parallel to the slide. The facial enamel surfaces were then ground on wet with 800, 1000, 1200, 2000, and 2500 grit silicone-carbide paper successively. On the day of experiments, teeth were stored in artificial saliva (kinhydrate, Spain) in 37ºC. After 30 minutes, they were subjected to Vicker's microhardness diamond at 50 g for 10 seconds. Three points were evaluated to obtain the mean microhardness. pH and titrable acidity of iron drop were measured which were respectively 2.13 and 1.5. After initial measures, the samples were randomly divided into three groups. The first group was immersed in iron drop (Ironorm, Wallace, England; BN 0B466) for five minutes at 37ºC. In the second group, a layer of silicone oil was rubbed on teeth before immersion in iron drop. In the third group, a layer of ACP-CPP paste was rubbed on tooth surface and left intact for three minutes.

Thereafter, all samples were immersed in iron drop for five minutes and microhardness was re-measured with the same mentioned method by a single blind observer. In addition, scanning electron microscopy (SEM) photos of one sample in each group and a polished mounted tooth without exposure to iron drop were taken for a qualitative comparison. Numeric data are expressed as means (standard deviation).

The mean differences between initial hardness and immersion within iron drop were calculated by means of One-way ANOVA. Homogeneity of variances was tested by Levene statistics. Changing trends of different study groups were assess using repeated measure general linear model (GLM). Data were analyzed with SPSS-18.

Results

Hardness of samples of all groups were significantly reduced by the time (p=0.001), yet no contrasting pattern was found between various groups (observed power=0.279). Mean observed differences of hardness for control, ACP-CPP and silicone oil groups were 111.6 (67.23), 67.38 (49.72) and 106.6 (73.21), respectively (Fig. 1). SEM photos were provided for qualitative analysis. As seen in figures 2 to 5, surface roughness after exposure to iron drop is evident, but the protective effect of ACP-CPP and silicone oil are not assessable by 2000 x magnification.

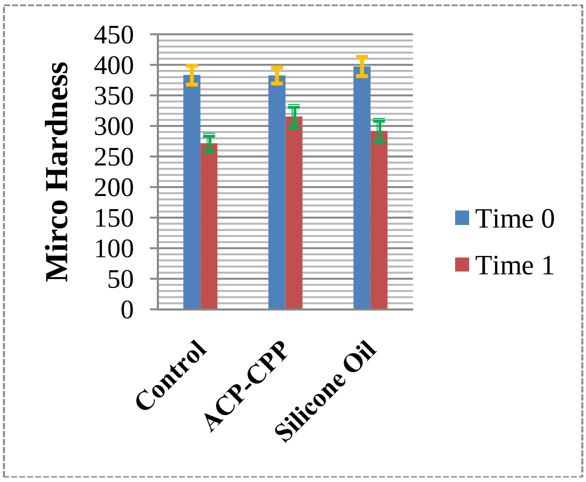

Figure1. Comparison of microhardness for various groups before (time 0) and after immersion in iron drop (Time 1)

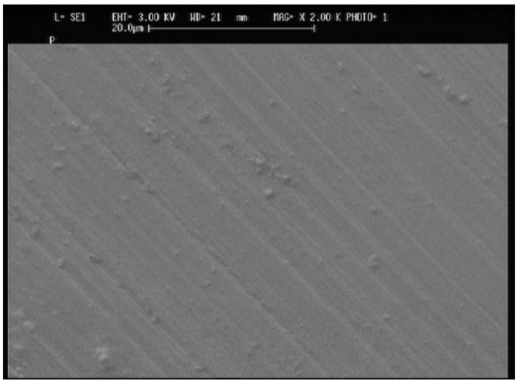

Figure 2. Polished surface of enamel without exposure to iron drop

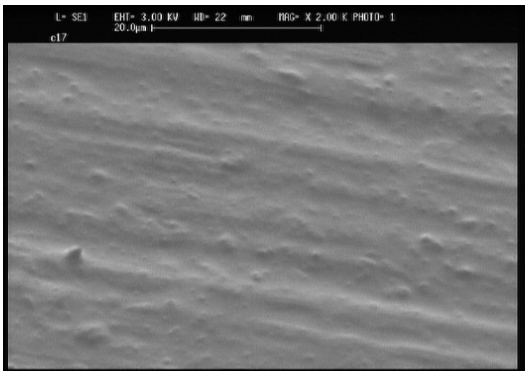

Figure 3. Polished surface of enamel after exposure to iron drop

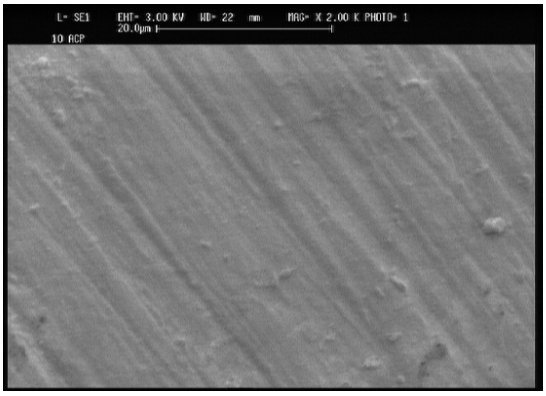

Figure 4. Polished surface of enamel after application of ACP-CPP and exposure to iron drop

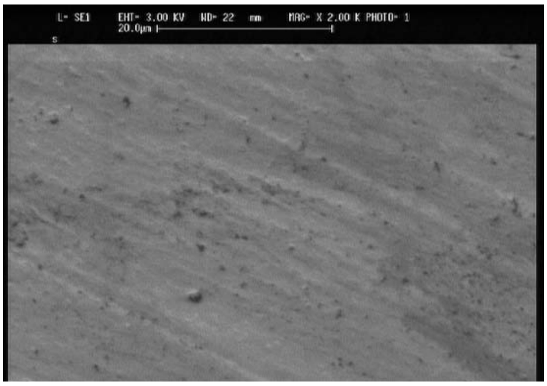

Figure 5. Polished surface of enamel without exposure to iron drop

Discussion

We found a significant reduction in microhardness of primary teeth after exposure to iron drop by Vicker's hardness test. Using none of the protective layers, ACP-CPP and silicone oil separately, could not prevent this significant reduction. Erosive effects of many drugs with low pH and high titrable acidity have been shown by microhardness measures and SEM photos in studies [11-13]. As iron drop also had a low pH and high Titrable acidity, this result was quite expected. By applying ACP-CPP on tooth surface before iron drop exposure, the reduction in microhardness was lessened to a great degree yet still remained statically significant; while applying silicone oil on tooth surface lessened the microhardness reduction only minimally. As mentioned earlier and observed in SEM photos, erosion makes a roughened surface which predisposes the tooth to staining and plaque retention and subsequent caries. To the best of our knowledge, this is the first study to assess protection against erosive properties of iron drop in primary teeth. Amorphous calcium phosphate casein phosphopeptide is a well-known product that is introduced to enhance remineralization and inhibit demineralization of tooth surface with therapeutic values for white spots, hypomineralized enamel, tooth hypersensivity, and erosion [13-15]. ACP-CPP is reported to reduce demineralization and enhance remineralization. This is because casein can adjust to acidic environments. In acidic pH, ACP will separate from CPP, thus increasing salivary calcium and phosphate levels. CPP can stabilize the level of ACP in saliva by preventing precipitation of calcium and phosphate, and stabilize their level in saliva [14, 15]. In this study, the mean reduction of microhardness in ACP-CPP group was less than the control group (68 versus 110) but the difference was not significant. In other words, ACP-CPP could not make enough protective effect against tooth erosion. Darshanet al. assessed the microhardness changes after bleaching and application of ACP-CPP on permanent teeth [16]. They concluded that this paste causes an increase in microhardness of bleached enamel. Unlike most microhardness studies, including our study, these authors did not polish the enamel surface prior to microhardness measurement. Flattening and polishing may eliminate the more acid-resistant surface layer of enamel, but also provides a test surface with uniform composition and erosion pattern which facilitates the standardization of specimens [17]. ACP-CPP could remineralize the unpolished enamel surface to a significant degree, an effect that might not be obtained if the surfaces were polished. Our samples were the extracted primary teeth of children in north, Iran. Permanent teeth are less susceptible to tooth erosion than primary teeth. So they may gain a significant increase in microhardness by a lesser remineralizing effect. This issue is true when comparing our study to many other studies that used permanent teeth and obtained a significant remineralizing effect and increase in micro hardness [14-18]. Furthermore, some previous studies regarding microhardness chose bovine enamel as their sample. Some structural differences have been mentioned between human and bovine enamel which are of great value in microhardness and erosion studies. Human enamel is more compact than bovine enamel. Bovine enamel is more porous and has less mineral content [17]. We assume that, as a more porous structure facilitates acid penetration and dissolution of mineral content, it facilitates penetration and deposition of ACP-CPP compound and thus promotes its remineralizing effect. As the enamel thickness and structure in different races is said to be different, possible ethnical variations in samples may lead to different results.

Artificial saliva may contain minimal contents of fluoride. It is suggested that fluoride may counteract with ACP component of casein complex thus hampering the remarkable protection of ACP-CPP [19]. Zhang et al. [20] found a significant increase in microhardness of demineralized enamel after applying ACP-CPP.

In fact, they assessed the remineralizing effect of ACP-CPP. On the contrary, we assessed preventive capacity of ACP-CPP against demineralization. We had two reasons for preferring the latter effect. In practice, when a child experiences the unfavorable taste of iron drop, he or she will not cooperate for subsequent application of ACP-CPP paste on teeth. The second reason was the assumption that, if iron drop erodes the unprotected tooth surface, it facilitates deposition of iron in subsurface layers. Later, even if ACP-CPP paste remineralizes the surface and compensates the mineral loss, it is of less value in prevention of staining and it may even make stain removal more difficult. However, it must be noted that as the application of ACP-CPP and iron drop consumption are clinically supposed to be repeated daily if suggested, CPP ACP would also have a remineralizing effect for the next cycle with a 24 hour interval. As keeping the paste intact in young children of 6 to 24 months (iron drop target group) is not an easy task, we chose three minutes, which is the least time of keeping the paste intact, reported to be useful in previous studies [21]. In Zhang study, ACP-CPP was left intact on teeth for five minutes, twice daily for 30 days. Although most in vitro studies are in favor of CPP ACP, at the interest of our study, in a systematic review by Azarpazhooh et al. regarding the clinical efficacy of casein derivatives, it was concluded that " the quantity and quality of clinical trial evidence are insufficient to make conclusions reagarding the long-term effectiveness of casein derivatives, specially ACP-CPP, in preventing caries in vivo and treating dentin hyper sensitivity" [21]. This result indicates that even if ACP-CPP showed effectiveness against iron drop erosion, there would still be a long way before its clinical application approval. Polydimethylsiloxane (silicone oil), has been a subject of study in preventive dentistry. It has an extremely low surface tension so it can spread over solid surfaces and form a tenacious hydrophobic, water repellent film which binds to hydroxyl apatite and changes the enamel properties [10]. It has shown benefits in keeping hydrophobic antibacterial compounds on tooth surface for longer periods of time [22].

In the present study, we tended to use silicone oil as a protective coating material. Oily material can make enamel more resistant to decalcification [23]. This effect may be contributed to water repellency and thus the material dissolved in water. As iron drop is a hydrophilic compound, we assumed that a water repellent layer can make a physical barrier against its contact with the tooth surface, and prevent the erosive effect and subsequent staining. However, the results were unlike what we expected. The specimens in silicone oil group had a significant reduction in microhardness after immersion in iron drop (-106), which was not very different from the control group (-110). We assume that immersion in iron drop might have washed away silicone oil. Boyer et al did not find any difference in caries lesion score by using silicone oil in rats either [24].

There is the possibility that salivary flow in the mouth will not be able to wash away the protecting layer before iron drop. Thus, further studies which simulate the salivary flow may be of value. As a limitation to this study, we did not measure the microhardness values in 3 stages: before the experiment, after exposure to iron drop, and after applying ACP-CPP. As described before, the latter methodology is considered for remineralizing effect. As for prevention of demineralization, we had to try the protective agents on a different group prior to applying ACP-CPP. Moreover, microhardness analysis does not have adequate accuracy to reveal subtle changes in demineralization. SEM photos by 2000x magnification did not demonstrate a detectable change in surface topography between groups. SEM photos of higher magnifications which reveal hydroxy apatite structure and more accurate microhardness tests may be required.

As for erosion, roughness test may be a better alternative. As there are differences in enamel both in structure and thickness in different affinities [25], different findings may be obtained by collecting samples from other ethnics. Clinical conditions including the changes in salivary flow rate and consistency after consuming the iron drop, which dilutes both iron drop and protective substances along with child resistance to drop ingestion which adds the abrasive effect of lips on tooth surface and frequency of drop consumption, are all worthy factors to be considered in further studies. Within the limitations of this study, iron drop showed an erosive effect on primary enamel surfaces and neither ACP-CPP, nor silicone oil could make a protective barrier against this erosive effect. Considering the vital effect of iron drop on child development and its deleterious effect of erosion on tooth surface and repeated complaints of staining, further studies in this field are strongly suggested.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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