THE EFFECT OF HIGH CALCIUM MILK AND CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE ON ENAMEL EROSION CAUSED BY CHOLINATED WATER

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Abstract. The aim of this study was to determine the effectiveness of high calcium milk and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on enamel erosion caused by chlorinated water. Thirty-six bovine enamel samples without wear or caries 3x4 mm in size were placed in acrylic blocks. All specimens were randomly allocated into 3 groups (n=12/group): CPP-ACP in the form of paste, Anlene ™ concentrated milk and a control (no treatment). All specimens were soaked in chlorinated water (pH =5.0) at room temperature for 72 hours following by soaking in artificial saliva for 30 minutes. Then, microhardness was determined using a microhardness tester. Data were analyzed using a one-way ANOVA and paired t-test. The microhardness value change in the control group was significantly higher than the other groups. No significant differences were seen between the 2 study groups. High calcium milk and CPP-ACP enhanced remineralization of enamel erosion caused by chlorinated water.

Key words: casein phosphopeptide- amorphous calcium phosphate, erosion, microhardness, milk

INTRODUCTION

Dental erosion is defined as a loss of dental hard tissue caused by acid, without bacterial involvement. Erosion may be caused by intrinsic or extrinsic factors. The intrinsic factors causing dental erosion include vomiting, gastroesophageal reflux, and rumination. The extrinsic factors are related to acidic environment, diet, medication and lifestyle (Zero, 1996; Magalhaes *et al*, 2007).

There have been several case reports of competitive swimmers suffering dental erosion from swimming in gas-chlorinated pools. Large swimming pools generally use gas chlorination for disinfection. The chlorine gas reacts with water to form hydrochlorous acid and hydrochloric acid. Hydrochlorous acid is the germicidal agent in chlorination. Hydrochloric acid is an unwanted by-product. Excess acid is usually neutralized and buffered through the addition of soda ash to maintain the recommended pH range of 7.2-8.0. Inadequate monitoring may result in a low pH.

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Chloridated pool water with a low pH (below 5.5) demineralizes human enamel; a significant correlation was found between a low pH in gas-chlorinated swimming pool water and dental erosions (Centerwall *et al*, 1986; Geurtsen, 2000; Caglar *et al*, 2005).

Dental erosions may cause tooth sensitivity and loss of the occlusal vertical dimension. The treatment of serious cases can be difficult and expensive. Therefore, it is important to prevent these erosions (Gabai *et al*, 1988).

Reducing tooth erosions involves removing the cause and the factors that enhance it. Preventive factors include enhancement of acid resistance and remineralization of the teeth, which requires calcium, phosphate and fluoride (Imfeld, 1996).

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) derived from bovine milk has been reported to reduce demineralization of the teeth and enhance remineralization (Reynolds, 1987, 1998; Reynolds *et al*, 2003). This effect occurs because casein, can adjust to acidic environments (Reynolds, 1987). At an acidic pH, amorphous calcium phosphate (ACP) will separate from casein phosphopeptide (CPP), thereby increasing salivary calcium and phosphate levels. CPP can stabilize the level of ACP in the saliva by preventing precipitation of calcium and phosphate and stabilizing the level of calcium and phosphate in saliva (Reynolds, 1998, 2003; Cross *et al*, 2004).

Bovine milk has anticariogenic properties in animal caries models and remineralizes enamel subsurface lesions *in vitro* (Reynolds, 1998). The anticariogenic properties of milk have been attributed to the presence of casein, calcium and phosphate (McDougall, 1977; Mor and Rodda, 1983; Reynolds *et al*, 2003). The majority of casein, calcium and phosphate ions in milk are bound in micelles. The size and decreased availability of ions in the micelles could theoretically limit the potential for milk to promote remineralization of enamel subsurface lesions *in situ*. We hypothesized bovine milk could enhance remineralization.

The aim of this study was to compare the effects of high calcium milk and CCP-ACP *in vitro* on enamel erosions caused by chlorinated water.

MATERIALS AND METHODS

Specimen preparation

Thirty-six bovine teeth without wear or caries were used in this study. Enamel specimens were cut from labial surfaces of the teeth and embedded in self-curing acrylic resin producing specimens measuring 3x4x3 mm. Specimens were then polished flat using 400, 800, 1,000, 1,200 and 2,500 grit silicon carbide sandpaper progressively (Buehler, Lake Bluff, IL) using a rotating polishing machine (Grinder-Polisher, Metaserv 2000 Buehler, West Yorkshire, UK) and stored in deionized water at room temperature until use. Baseline surface hardness of sound enamel was measured with the Vicker indenter tester (FM-700e Type D, Future-Tech, Tokyo, Japan) using 100 g of force for 15 seconds. Four indentations per test were performed on each specimen and calculated by averaging the value of the four indentations.

Erosion prevention procedure

All specimens were divided randomly into four groups of 12 teeth each: the teeth in the groups were treated as follows: a 0.5 mm layer of CPP-ACP was applied to the enamel surface for 5 minutes in group 1, the specimens were immersed in bovine

Table 1
Materials used in this study.

Material	Manufacturer	Composition
Casein Phosphopeptide-amorphous calcium phosphate (Tooth mousse)	GC, Tokyo, Japan	Pure water, glycerol, casein Phosphopeptide- amorphous calcium phosphate, D-sorbitol, silicon dioxide, sodium carboxymethylcellulose, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl-*p*-hydrobenzoate, prophy-*p*-hydrobenzoate.
Anlene™ milk	Dairy Plus Co Ltd, Nakhon Sawan, Thailand	Skim milk, whole milk, concentrated skim milk, vegetable gums, lactose, minerals (calcium, magnesium, zinc), vitamin D

Table 2
Microhardness at baseline and after the erosion procedure (N=12).

Enamel treatment	Group (Mean VHN± SD)		
	CPP-ACP	Anlene™ concentrate milk	No treatment (control group)
Baseline	306.27+8.76	302.35+7.89	306.08+7.71
After erosion procedure	306.08+10.08	297.21+7.99	268.08+17.65[a]

[a]Siginificant different from the other groups.

milk for 5 minutes in group 2, and control specimens in group 3 received no treatment (Table 1).

Erosion procedure

All specimens were soaked in chlorinated water at a pH of 5.0 at room temperature for 72 hours, then immersed in artificial saliva for 30 minutes. All specimens were then rinsed in deionized water and blot dried. The Vicker indenter test was performed using 4 indentations, at least 50 μm apart, and the average of the 4 indentations was used as the microhardness value.

Statistical analysis

Since all variables tested satisfied assumptions of equality and normal distribution, the one-way ANOVA and paired t-tests were used to compare changes in enamel microhardness, at a 95% confidence level.

RESULTS

The mean baseline microhardness values were 270-320 KHN. No significant statistically differences were seen among groups at baseline.

After the erosion procedure the microhardness value in the control group had a significantly greater decrease compared to the study groups (Table 2).

DISCUSSION

Baseline microhardness values were 270-320 KHN. These values are similar to previous studies (Meredith *et al*, 1996; Maupome *et al*, 1998). Our study design required a sufficiently flat area to allow microhardness measurements, thus the area subjected to erosion was not the origin surface enamel. Microhardness decreases from the outer enamel surface toward the dentinoenamel junction (Meredith *et al*, 1998), which may explain the range in baseline values.

After the erosion procedure, the microhardness of the teeth decreased siginificantly in the control group. The microhardness decreased 0.003% in the CPP-ACP group and 1.65% in the Anlene™ concentrate milk. This is consistent with the findings of many other studies that demonstrated the remineralization effects of CPP-ACP and milk (Shen *et al*, 2001; Reynolds 2003; Walker *et al*, 2006; Cai *et al*, 2007; Rahiotics and Vougiouklakis, 2007; Tantibirojn *et al*, 2008).

CPP-ACP was observed to reduce erosive enamel wear under severe erosive conditions simulating a combination of heavy attrition and gastric regurgitation. Previous studies showed the potential of CPP-ACP as an anticariogenic agent both *in vitro* and *in vivo* (Reynolds, 1997, 2003; Kumar *et al*, 2008) with CPP-ACP preventing demineralization and promoting remineralization of subsurface carious lesions in enamel and dentine (Rahiotis and Vougiouklakis, 2007). However, the mechanism by which CPP-ACP reduces erosion is unclear (Ramalingam *et al*, 2005; Ranjitkar *et al*, 2009). A recent study showed CPP-ACP can increase microhardness of enamel and reduce erosions by cola drinks (Tantbirojn *et al*, 2008), implying CPP-ACP is capable of remineralizing eroded lesions. The process of remineralization of eroded lesions is unclear, but it is likely to involve deposition of mineral into the porous zone rather than crystal regrowth (Eisenburger *et al*, 2001).

Anlene™ is a high calcium milk which contains 4 times the calcium as regular milk. Two hundred milliliters of regular milk provides 220 mg of calcium or 1.1 mg per ml. One hundred ten milliliters of Anlene™ contains 500 mg of calcium or 4.5 mg per ml. Previous studies have shown that bovine milk possesses anticariogenic properties in animal caries models (Reynolds and Cai, 1999; Morgan *et al*, 2008) and can remineralized enamel subsurface lesions *in vitro* (McDougall 1977; Mor and Rodda, 1987). The anticariogenic properties of milk have been attributed to the presence of casein, calcium and phosphate (McDougall, 1977; Mor and Rodda, 1983; Reynolds *et al*, 2003). It contains approximately 30 mM of calcium, of which only 10 mM is not bound in casein micelles and only 2 mM is in the form of free calcium ions (Neville *et al*, 1994; Tanaka *et al*, 1999). Whilst the calcium in casein micelles is unlikely to diffuse across the relatively intact enamel surface layer into the subsurface defect, the free calcium ions in milk should be bioavailable. This was confirmed by this current study, which found that Anlene™ milk remineralized enamel subsurface erosions *in vitro*. High calcium milk and CPP-ACP enhance remineralization of enamel erosion caused by chlorinated water.

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