EFFECTIVENESS OF SOY MILK WITH CALCIUM ON BOVINE ENAMEL EROSIONS AFTER SOAKING IN CHLORINATED WATER

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Abstract. We determined the effectiveness of soy milk with calcium for prevention of enamel erosion caused by chlorinated water. Thirty-six bovine teeth without wear or caries, sized 8x10 mm, were placed in resin acrylic blocks. All specimens had an initial enamel microhardness of 270-320 VHN and were randomly allocated into 3 groups (n=12/group): control (not treated), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) tooth mousse and soy milk with calcium. We applied the CPP-ACP for 5 minutes or soaked the teeth in soy milk with calcium for 20 minutes. Then, all the specimens were soaked in chlorinated water (pH 5.0) at room temperature for 96 hours following by 30 minutes in artificial saliva and then the microhardness of each tooth was re-determined. The data were analyzed statistically using the one-way ANOVA test and the paired t-test. The group with the least reduction in microhardness was the CPP-ACP treated group, followed by the control group and then the soy milk with calcium treated group. The enamel microhardness value after exposure to chlorinated water in the soy milk with calcium group was not significantly different from the control group ($p>0.05$). The CPP-ACP treated group was significantly different from the other 2 groups ($p<0.05$). Soy milk with calcium gave no protection against dental erosions caused by chlorinated water.

Keywords: calcium, chlorine water, dental erosion, soy milk, tooth wear

INTRODUCTION

Large swimming pools generally use chlorine gas to disinfect the water. The pH of the water is then adjusted to about 7.5 by the addition of acid or alkali (Dawes and Boroditsky, 2008). If the pH is not neutralized, usually with sodium carbonate, it can drop to 3 or even lower (Centerwall *et al*, 1986; Geurtsen, 2000). Several studies have found an increased prevalence of dental erosions among frequent swimmers due to the low pH of gas-chlorinated pool water (Geurtsen, 2000; Caglar *et al*, 2005).

The treatment of dental erosions is difficult and expensive. Therefore, prevention is the best option (Gabai *et al*, 1988).

Some studies report less severe tooth

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erosions by removing the cause or reducing factors that enhance it (Imfeld 1996; Manton *et al*, 2010). Tooth erosion preventive factors include enhancement of acid resistance by the tooth and remineralization, 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 tooth structures and enhance remineralization by delivering calcium and phosphate to the tooth enamel in a soluble form, enabling remineralization of the enamel (Reynolds, 1998; Reynolds *et al*, 2003).

Mor and Rodda (1983) found bovine milk remineralized enamel subsurface lesions *in vitro*. The remineralization properties of milk have been attributed to the presence of casein, calcium and phosphate (Mor and Rodda, 1983; Reynolds *et al*, 2003). It was hypothesized bovine milk enhances tooth remineralization capacity.

Many people drink soy milk instead of bovine milk. A common reason for this is bovine milk allergy. Soy milk is not a very good source of calcium in its natural state, but many manufacturers add calcium to soy milk. No studies have reported remineralization effect of soy milk enriched with calcium on dental erosions caused by chlorinated water.

We compared the effect of soy milk enriched calcium against CCP-ACP to prevent enamel erosions *in vitro* caused by chlorinated water.

MATERIALS AND METHODS

Specimen preparation

Thirty-six bovine teeth without wear or caries were used for this study. The enamel specimens were cut from labial surfaces of bovine teeth and embedded in self-cured acrylic resin producing specimens measuring 3x4x3 mm. The specimens were then polished flat with 400, 800, 1,000, 1,200 and then 2,500 grit silicon carbide sandpaper (Buehler, Lake Bluff, IL) with a rotating polishing machine (Grinder-Polisher, Metaserv 2000; Buehler; Lake Bluff, IL) and stored in deionized water at room temperature until used. Baseline surface hardness of the sound enamel was measured with a 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 then the average of the measurements was calculated.

Preventive erosion process

The specimens were divided randomly into 3 groups of 12 teeth each. Group 1 specimens were treated with a 0.5 mm layer of CPP-ACP on the enamel surface for 5 minutes. Group 2 specimens were immersed in soy milk enriched with calcium for 5 minutes. Group 3 specimens received no treatment (control). A description of the treatment materials used for the study is shown in Table 1.

Erosion process

All specimens were soaked in chlorinated water, pH 5.0, at room temperature for 72 hours and then immersed in artificial saliva for 30 minutes. The specimens were then rinsed with deionized water and blotted dry. A Vicker indenter tester was used to cause four indentations, at least 50 m apart, to determine the microhardness of the teeth. The average of the 4 indentation was used for the final value.

Statistical analysis

Since all the variables tested satisfied assumptions of equality and normal distribution, one-way ANOVA and paired

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 carboxymethy cellulose, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl-*p*-hydrobenzoate and prophy-*p*-hydrobenzoate
Nature's soy enriched with calcium	Pureharvest, Victoria, Australia	Water, organic whole soy beans, organic sunflower oil, mineral salts (calcium carbonate, ferrous sulphate)

Table 2
Microhardness values at baseline and after the erosion process (n=12).

Group	Condition (Mean VHN±SD)	
	Baseline	After erosion process
CPP-ACP	319.33 ± 13.05^{Aa}	316.96 ± 10.71^{Ba}
Soy milk with calcium	311.97 ± 17.68^{Aa}	251.75 ± 24.94^{Cb}
No treatment (control group)	316.33 ± 6.40^{Aa}	268.04 ± 17.65^{Cb}

Within columns, different upper-case superscript letters indicate significant differences among treatment groups (one-way ANOVA, $p<0.05$).
Between columns, different lower-case superscript letters indicate significant differences by condition (one-way repeated measures ANOVA, $p<0.05$).
VHN, Vickers hardness number.

t-tests were used to compare enamel microhardness changes with 95% confidence levels.

RESULTS

The mean baseline microhardness values ranged from 270 to 320. There were no significant differences in hardness at baseline among groups.

Teeth treated with calcium enriched soy milk had the greatest decrease in microhardness, significantly lower than teeth treated with CPP-ACP group (Table 2).

DISCUSSION

The baseline microhardness values for enamel in this study ranged from 270 to 320 KHN, similar to previous studies (Meredith *et al*, 1996; Vongsawan *et al*, 2010). Our study design required a sufficiently large flat area to test microhardness, so the original enamal surface was not used.

In this study, microhardness decreased siginificantly in both the soy milk and control groups. The microhardness decreased, significantly greater than the decrease in the CPP-ACP group.

CPP-ACP can increase tooth micohardness and reduce erosions caused by chlorinated water (Vongsawan *et al*, 2010; Rirattanapong *et al*, 2011). The mechanism by which CPP-ACP reduces erosions is unclear (Ranjitkar *et al*, 2009). It is likely to involve a repair process by deposition of mineral into the porous zone rather than crystal regrowth (Eisenburger *et al*, 2001).

A study by de Mazer Papa *et al* (2010) reported soy-based formula had the potential to induce demineralization in primary enamel. Soy milk, made from soybeans, soaked, boiled, ground up and filtered, is a good source of protein but is low in fat and carbohydrates. The carbohydrate in soy milk is sucrose, the same carbohydrate found in sugar cane. The largest downfall of soy milk is the lack of calcium. It has only about one quarter the calcium of bovine milk. Many soy milk manufacturers add calcium to their products, but studies show this is not as healthful as naturally-occuring calcium (Narula, 2009).

Soy milk enriched with calcium has no remineralizing effect on enamel erosions caused by chlorinated water.

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