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An in situ caries study on the interplay between fluoride dose and concentration in milk

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ABSTRACT

Objectives: This randomized, cross-over in situ study investigated the impact of sodium fluoride dose and concentration in milk on caries lesion rehardening, fluoridation and acid resistance.

Methods: Twenty-eight subjects wore two gauze-covered enamel specimens with pre-formed lesions placed buccally on their mandibular partial dentures for three weeks. Participants used fluoride-free dentifrice throughout the study and consumed once daily one of the five study treatments: no fluoride in 200 ml milk (0F-200), 1.5 or 3 mg fluoride in either 100 (1.5F-100; 3F-100) or 200 ml milk (1.5F-200; 3F-200). After three weeks, specimens were retrieved. Knoop hardness was used to determine rehardening and resistance to a secondary acid challenge. Enamel fluoride uptake (EFU) was determined using a micro-biopsy technique.

Results: A linear fluoride dose–response was observed for all study variables which exhibited similar overall patterns. All the treatments resulted in rehardening, with 0F-200 inducing the least and 3F-100 the most. Apart from 1.5F-200, all the treatments resulted in statistically significantly more rehardening compared to 0F-200. The fluoride doses delivered in 100 ml provided directionally although not statistically significantly more rehardening than those delivered in 200 ml milk. EFU data exhibited better differentiation between treatments: all fluoridated milk treatments delivered more fluoride to lesions than 0F-200; fluoride in 100 ml demonstrated statistically significantly higher EFU than fluoride in 200 ml milk. Findings for acid resistance were also more discerning than rehardening data.

Conclusions: The present study has provided further evidence for the anti-caries benefits of fluoridated milk. Both fluoride dose and concentration appear to impact the cariostatic properties of fluoride in milk.

1. Introduction

The delivery of cariostatic amounts of fluoride through milk is not novel by any means as the first caries studies in children were conducted in the 1960s.[1] Despite its prolific history, a Cochrane Systematic Review[2] concluded 'that there is insufficient evidence to show the effectiveness of fluoridated milk in preventing tooth decay' and highlighted the need for further randomized clinical trials. Yet, milk fluoridation has been shown to be an effective public health measure and more recent studies have provided further supporting evidence.[1,3] Milk fluoridation has been recommended where fluoride concentration in the drinking water is suboptimal, for target

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groups with high caries prevalence and poor compliance for oral hygiene, in areas without or suboptimal water fluoridation and where school- or kindergarten-based programmes to provide milk to children are already in place.[3,4]

Recent research has focused on determining if fluoride in milk follows a dose–response pattern and if an optimum fluoride concentration exists. However, results of laboratory [5–7] and in situ studies[8,9] into the anti-caries effects of different milk fluoride concentrations have been somewhat equivocal – benefits of fluoridated vs. non-fluoridated milk have been reported unanimously in vitro and in situ; although a clear fluoride dose–response relationship has yet to be established in situ. Likewise, there appears to be some disagreement with regards to an optimum fluoride concentration in milk as results obtained using a range of laboratory models led authors to different conclusions.[5–7]

Furthermore, the cariostatic properties of fluoride do not only depend on dose but also on concentration.[10] While drawing parallels to conventional fluoride delivery vehicles, such as dentifrices and rinses, is not straightforward, especially considering their much higher fluoride concentrations in comparison to milk, a study on rinses demonstrated that, for a given fluoride dose, the main driving force for efficacy was fluoride concentration; i.e. rinsing with a smaller volume but higher fluoride concentration was more beneficial than rinsing with a larger volume but lower fluoride concentration.

Therefore, the aims of the present in situ study were threefold: (a) the primary objective was to determine if a higher dose of fluoride in milk would provide a greater caries preventive effect as determined by measuring early caries lesion rehardening, fluoridation and acid resistance; and (b) the secondary objectives were to determine if a higher concentration of fluoride in milk would provide a greater caries preventive effect and if the caries preventive effect of fluoridated milk follows a dose–response pattern.

2. Materials and methods

2.1. *Ethical aspects*

The study protocol was reviewed and approved by the IUPUI Institutional Review Board, #1206008830. It was conducted at the Oral Health Research Institute of the Indiana University School of Dentistry. All subjects signed a written informed consent prior to screening and received oral soft and hard tissue examinations throughout the study.

2.2. *Experimental design*

The study was randomized, investigator-blind, observer-blind, laboratory analyst-blind, and utilized a 5-way cross-over design. Subjects were partially blind to the treatments (labelled A–E) as they consumed two different milk volumes throughout the study. Two to three days following a dental cleaning, two partially demineralized specimens were placed in the buccal flange area of the subject's mandibular partial denture. Specimens were individually wrapped in Dacron gauze to facilitate plaque growth. Wrapped specimens were mounted in close proximity to each other and flush with the denture surface. Subjects were instructed on the milk preparation (see below), consumed the first treatment under supervision at the study site and received a diary for home use which they returned at the end of each treatment period. During each of the five, three-week test periods, subjects drank their assigned milk test product after dinner (in the evening), once per day for either five (100 ml dose) or ten (200 ml dose) timed minutes, wearing their mandibular partial dentures 24 h a day during the test period including during meals. Dinnertime was chosen to avoid interfering with the subjects' lifestyles and to maximize the cariostatic benefits of fluoride.[11] Subjects used fluoride-free dentifrice (Natural Tea Tree Oil Toothpaste, Desert Essence, NY, USA) two to three days before and continuously during each treatment period. This choice was made to mimic high-risk populations, although subjects were exposed to fluoridated water (approx. 1.0 ppm) during the study. At the end of each three-week test period, subjects returned to the study site, specimens were removed and analyzed. All subjects received a professional fluoride treatment (APF Gel, PediaGel, Preventech, NC, USA) at the end of the study. The main response variables were percent surface microhardness recovery (%SMHr), enamel fluoride uptake (EFU), and percent acid resistance (%AR) measured on the enamel experimental specimens. Each subject served as his or her own control.

2.3. *Power calculation*

Based on prior studies using a variety of conventional oral care products in this model [unpublished data], the within-product standard deviation of %SMHr is estimated to be 13% and the correlation between products is expected to be approximately 0.5. With a sample size of 28 subjects in a 5-way cross-over study, the study had 80% power to detect a %SMHr difference of 8.6%, assuming two-sided tests each conducted at a 5% significance level.

2.4. *Enamel specimens and lesion creation*

Specimens obtained from human permanent teeth were used as the hard tissue test substrate. The teeth were collected and transported to OHRI in a saturated thymol solution. Upon receipt, the teeth were sorted, cleaned and the root tips removed. The teeth were then stored in saturated thymol solution during sample preparation procedures. Teeth were selected based on the following criteria: free of caries and major restorations; no discoloration and no markings, such as cracks, when viewed under a microscope at 20× magnification; sufficient tooth surface to provide a large size specimen to meet study requirements.

Up to two specimens were obtained from the buccal and/or lingual smooth surface of each tooth. Longitudinal sections approximately 3 mm in thickness were made parallel to the selected tooth surfaces. The tooth sections were then cut into 4 mm × 4 mm specimens using a Buehler Isomet low-speed saw. Specimens were ground and polished to create planar parallel dentine and enamel surfaces. The dentine side was ground flat using 500 grit silicon carbide paper, followed by grinding and polishing of the enamel side. A small orientation

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