COMPARISON OF MILK AND DESENSITIZING DENTIFRICES IN REDUCING HYDRAULIC CONDUCTANCE OF HUMAN DENTIN IN VITRO

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Abstract. Dentin hypersensitivity is a common condition usually associated with exposed dentinal surfaces which can become a problem especially in elderly people. Hydraulic conductance of dentin has been used to evaluate the sensitivity of dentinal tubules. The aim of this study was to compare the effects of cow’s milk, two desensitizing dentifrices and 3% potassium tetraoxalate (positive control) in reducing the hydraulic conductance of human dentin in extracted teeth. We transversely sectioned 28 non-carious extracted human premolars 2 mm below the cemento-enamel junction. We exposed the dentin at the tip of the buccal cusp by cutting a cavity (3 mm diameter, 3 mm deep), and examined the hydraulic conductance of the dentin using the fluid filtration device under 100 mm Hg hydrostatic pressure. We removed the smear layer before each test using 37% phosphoric acid for 30 seconds. Each tooth was examined after being treated with the tested substance. Each substance was examined for each tooth. The substances examined were: cow’s milk, Colgate Sensitive Pro-relief\textsuperscript{TM}, Sensodyne\textsuperscript{®} Rapid Relief, and 3% potassium tetraoxalate. The dentinal tubules were also examined with a scanning electron microscope. Milk, Colgate Sensitive Pro-relief\textsuperscript{TM}, Sensodyne\textsuperscript{®} Rapid Relief, and 3% potassium tetraoxalate significantly reduced hydraulic conductance by 15.0%, 18.6%, 17.5%, and 72.6%, respectively, from their baseline values ($p<0.05$, paired $t$-test). No significant differences were seen between cow’s milk and Colgate Sensitive Pro-relief\textsuperscript{TM} and Sensodyne\textsuperscript{®} Rapid Relief ($p>0.05$). Cow’s milk reduced hydraulic conductance to a similar degree to the selected desensitizing dentifrices. Cow’s milk may be an alternative choice to treat patients suffering from dentin hypersensitivity.

Keywords: dentin permeability, dentin sensitivity, desensitizing dentifrices, human dentin, hydraulic conductance, milk

INTRODUCTION

Dentin hypersensitivity is a common clinical problem usually associated with exposed cervical dentin surfaces. It is characterized by short, sharp pain arising from the response of exposed dentin to stimuli, typically thermal, evaporative, tactile, osmotic or chemical, which cannot be ascribed to any other dental defect or pathology (Holland et al, 1997). The prevalence of dentin hypersensitivity in the general population ranges between 34% and 52% (Gillam et al, 1999; Wang
et al, 2012; West et al, 2013), and 68-84% among patients with periodontal disease (Chabanski et al, 1996; Rees et al, 2003). Dentin can become exposed with loss of enamel and cementum following gingival recession. Patients with cervical exposed dentin may not immediately develop dentin hypersensitivity, because the dentin may be covered by a smear layer that occludes the exposed dentinal tubules (Orchardson, 2000; Pashley, 2013). In these patients, no pain or only mild pain is felt when their teeth are exposed to stimuli such as an air blast or tooth-brushing. The more appropriate term used for these cases is “dentin sensitivity”. The term “dentin hypersensitivity” is used when these patients described a significant increase in their sensitivity to such stimuli (Pashley, 2013). One mechanism for dentin hypersensitivity is consuming an acidic diet (Absi et al, 1987; Kijsamanmith et al, 2016). An acidic diet can remove the smear layer and leave open dentinal tubules exposed to the oral environment. An air blast or probing can cause more fluid to flow through the open dentinal tubules if there is no smear layer. The fluid shift excites the nerves in the inner end of the dentinal tubules or in the pulp. The increased hydraulic conductance of the dentin can result in pain, supporting the hydrodynamic theory of dentin sensitivity (Brännström, 1963; Charoenlarp et al, 2007; Vongsavan and Matthews, 2007).

Desensitizing toothpastes, such as 8% strontium acetate and 8% arginine/calcium carbonate toothpastes were developed to reduce the pain of dentin hypersensitivity. The mechanism of these toothpastes is to occlude the exposed dentinal tubules, leading to decreased hydraulic conductance of the dentin (Cummins, 2010; Mason et al, 2010; Davies et al, 2011; Seong et al, 2013). Strontium-based paste is believed to occlude dentinal tubules by substituting the calcium ion with strontium ion in the hydroxyapatite crystals in the dentinal tubules (Kun, 1982). Arginine/calcium carbonate paste has been shown to plug dentinal tubules (Davies et al, 2011; Seong et al, 2013). This is probably due to the formation of a negatively charged agglomeration of arginine and calcium carbonate, which binds to the dentinal tubules (Kleinberg, 2002).

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is also used to occlude dentinal tubules (Walsh, 2010). Konekeri et al (2015) reported CPP-ACP has a greater efficacy than potassium nitrate in reducing dentin hypersensitivity to tactile stimulation. Cow’s milk is a rich source of CPP-ACP (Reynolds et al, 2003). Cow’s milk may be useful in reducing the hydraulic conductance of dentin.

A fluid filtration technique has been developed to compare the hydraulic conductance of dentin with different treatments on the same dentin of the crown from extracted teeth (Kijsamanmith et al, 2016). This technique allows comparison of different treatments on the same exposed dentin to be evaluated.

The objective of the present study was to determine the effect of cow’s milk on reducing the hydraulic conductance of human dentin, compared with two desensitizing dentifrices: 8% arginine/calcium carbonate paste (Colgate Sensitive Pro-relief™) and 8% strontium acetate (Sensodyne® Rapid Relief), and 3% potassium tetraoxalate (positive control). The morphology of exposed dentin after treatment with these desensitizing agents was also investigated, to evaluate the capacity to occlude open dentinal tubules. The null hypothesis was that there was no difference in the ability of cow’s milk
or desensitizing dentifrices to reduce the hydraulic conductance of human dentin.

MATERIALS AND METHODS

This study was conducted among 28 extracted human premolars that were fully erupted, free of caries, without restorations that were extracted for orthodontics purposes. The teeth were kept in 0.9% saline solution containing amoxicillin (500 mg/l) and used within two weeks of extraction. The study protocol was approved by the Institutional Review Board, Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Bangkok, Thailand (COE. No. MU-IRB 2010/035.0709).

Tooth preparation

All 28 teeth were transversely sectioned 1-2 mm below the cemento-enamel junction using a diamond disc (Komet type 917, Lemgo, Germany) under water spray and the coronal pulp was removed. A cavity 3 mm in diameter and 3 mm in depth was prepared on the buccal cusp using a diamond bur (Intensive® No.204, Viganello-Lugano, Switzerland) in a high speed hand-piece under water spray. The exposed dentin was prevented from drying using normal saline solution.

Hydraulic conductance (Dentin permeability) measurement

Eighteen premolar crown specimens were used for the hydraulic conductance (dentin permeability) measurements. Hydraulic conductance was measured using the fluid filtration method (Kijsamanmith et al, 2016). Briefly, after tooth preparation, each crown section was glued with cyanoacrylate adhesive (Alteco, Osaka, Japan) to a plastic block containing a stainless steel tube (G18) that was connected to a glass capillary (internal diameter, 300 µm; DADE, Miami, FL). The pressure of the system was controlled by attaching the tubing from the capillary to a mercury manometer. The pulp chamber, capillary, and tubes were filled with normal saline solution. Fluid flow through the dentin was determined from the movement of a tiny air bubble introduced into the capillary (Fig 1).

Application of desensitizing agents and experimental design

The prepared cavities were etched with 37% phosphoric acid (Ivoclar Vivadent AG, FL-9494 Schaan, Liechtenstein) for 30 seconds to remove the smear layer created during the preparation procedure. Distilled water (negative control) was put
in the cavity (without the smear layer) for 5 minutes at a pressure of 11 mmHg (1.47 kPa) above atmospheric pressure to represent the normal pulpal tissue fluid pressure (Vongsavan and Matthews, 1992). The baseline fluid flow through the dentin was confirmed using a positive pressure of 100 mmHg (13.33 kPa). Each of the 18 teeth was subjected to all test materials by sequential random treatment with cow’s milk (pasteurized full fat milk, Meiji Brand, Saraburi, Thailand), toothpaste containing pro-argin (Colgate Sensitive Pro-relief™), and toothpaste containing strontium acetate (Sensodyne® Rapid Relief). Of the 18 tested specimens, each test agent had an equal chance of being used first in the order of treatment. For each treatment, the agent was applied to the cavity for 5 minutes with a pulp pressure of 11 mmHg above atmospheric pressure and the cavity was rinsed with distilled water for 15 seconds. Fluid flow through the dentin was confirmed with a positive pulpal pressure of 100 mmHg. Three consecutive measurements were taken and hydraulic conductance ($L_p$) was calculated as follows:

$$L_p = \frac{\text{flow rate/pressure}}{\text{area of dentin exposed}}$$

After each treatment, the treated dentin surface was re-etched with 37% phosphoric acid for 30 seconds to remove the sealing agent and reopen the dentinal tubules. Baseline hydraulic conductance was measured again before each treatment. With the last treatment, the fluid flow through the dentin was confirmed before and after treatment with 3% potassium tetraoxalate for 5 minutes.

After the experiments, all 18 specimens were sectioned longitudinally through the cavity. The remaining thickness of each tooth was measured between the floor of the cavity and the closest pulpal horn using calipers.

**Scanning Electron Microscopy (SEM)**

After tooth preparation and smear-layer removal, 10 specimens were randomly divided into 5 groups of 2 specimens each: group 1: no treatment; group 2: treated with cow’s milk; group 3: treated with toothpaste containing pro-argin; group 4: treated with toothpaste containing strontium acetate; and group 5: treated with 3% potassium tetraoxalate. All teeth were processed and examined using a scanning electron microscope (JSM-5410 LV; JEOL, Tokyo, Japan).

**Statistical analysis**

With each treatment, the mean hydraulic conductance and standard deviation (SD) at baseline and after treatment were measured and compared with the paired $t$-test. A $p$-value < 0.05 was considered statistically significant.

Reductions in the percentages of hydraulic conductance among the study groups were compared using the one-way, repeated measures analysis of variance (one-way RM ANOVA). The Tukey test was used to compare means.

**RESULTS**

The mean (±SD) hydraulic conductances of the teeth treated with the studied substances are shown in Fig 2. The cow’s milk and desensitizing dentifrices tested significantly reduced the hydraulic conductance of the human dentin in the extracted teeth. The mean post-treatment hydraulic conductance significantly decreased ($p < 0.001$) from baseline in all the treatment groups.

The mean (±SD) reductions in hydraulic conductance in the teeth treated
Fig 2—Mean values of the hydraulic conductance in 18 teeth treated with cow’s milk and different types of desensitizing agents. Data were recorded before (black column) and after (gray column) treatment with each agent. Each error bar represents 1 SD. Significantly lower values ($p < 0.001$) were found after treatment with each agent compared with pre-treatment. *Significant differences by paired $t$-test ($p < 0.001$). SD, standard deviation.

Fig 3—Percent reduction in hydraulic conductance by treatment group after 5 minutes. The same lowercase letter indicates no significant difference. SD, standard deviation.

with cow’s milk, toothpaste containing pro-argin, toothpaste containing strontium acetate, and 3% potassium tetraoxalate were 14.97 ($\pm 11.35\%$), 18.57 ($\pm 8.51\%$), 17.53 ($\pm 9.62\%$), and 72.56 ($\pm 8.64\%$), respectively. There were no significant differences among the tested treatment groups ($p > 0.05$) (Fig 3). Potassium tetraoxalate, used as a positive control, showed the greatest reduction in hydraulic conductance and was significantly different from the tested treatment groups ($p < 0.001$).

Scanning electron micrographs of the exposed acid-etched dentin showed a clean dentin surface with open dentinal tubule orifices. The dentin specimens treated with cow’s milk appeared to be covered by a thin precipitate on the dentin surface and some precipitates adhered to the periphery of the dentinal tubule orifices,
partially occluding them. The dentin specimens treated with either Colgate Sensitive Pro-relief™ or Sensodyne® Rapid Relief also showed partially occluded dentinal tubules, like those treated with cow’s milk. Among the teeth treated with 3% potassium tetraoxalate, most of the dentinal tubules were occluded with calcium oxalate crystals penetrating the dentinal tubules (Fig 4). The mean (±SD) remaining dentin thickness of the teeth used was 1.83 (±0.30) mm ($n=18$).
DISCUSSION

Our study cow’s milk decreased the hydraulic conductance of human dentin by 15%, which was not significantly different from the desensitizing dentifrice containing pro-argin (Colgate Sensitive Pro-relief™) or the toothpaste containing strontium acetate (Sensodyne® Rapid Relief), but significantly lower than 3% potassium tetraoxalate.

Cow’s milk contains high concentrations of protein, calcium, and phosphate (Aimutis, 2004). Dairy casein phosphopeptide (CPP) contains phosphoserine sequences, which bind to and stabilize particles of amorphous calcium phosphate (Reynolds, 1997). The stabilized CPP-ACP prevents the dissolution of calcium and phosphate ions and maintains a supersaturated solution of calcium and phosphate (Reynolds, 1997). Cow’s milk has been found to have remineralization potential both in vitro (McDougall, 1977) and in vivo (Featherstone and Zero, 1992). Our results show cow’s milk has remineralization potential and reduces dentin permeability and dentin hypersensitivity.

The desensitizing dentifrices tested in our study decreased hydraulic conductance. Colgate Sensitive Pro-relief™ toothpaste contains the amino acid arginine, which works in conjunction with calcium carbonate and phosphate to create a plug in the dentinal tubules on the exposed dentin, preventing fluid flow in the dentin, thereby reducing dentin hypersensitivity (Cummins, 2010; Petrou et al, 2009). Sensodyne® Rapid Relief containing 8% strontium acetate and 1,040 ppm sodium fluoride has remineralizing potential due to its ability to be incorporated into the mineral phase of enamel by replacing calcium within the apatitic lattice (Saleb and Debruyn, 1972) and has tubule occluding properties due to the presence of an artificial silica abrasive, which when available in a non-ionic detergent base, as in this product, has a strong affinity for dentin and resists removal by acidic solutions (Banfield and Addy, 2004).

Olley et al (2012) found after 2 days of treatment in vivo brushing twice daily, 8% strontium acetate and 8% arginine both had significantly greater dentin occlusion than the control dentifrice (1,450 ppm sodium fluoride) and water, similar to our study.

The greatest reduction in hydraulic conductance in our study was in the 3% potassium tetraoxalate group. Previous studies have found that potassium oxalate decreases hydraulic conductance of dentin by up to 98% (Pashley and Galloway, 1985; Pashley, 1986). Oxalate reacts with ionized calcium in the dentinal tubules, forming crystals of insoluble calcium oxalate that can partially occlude the tubule 5-7 µm below the surface (Greenhill and Pashley, 1981). In our study, scanning electron-microscopy revealed crystals of calcium oxalate in the dentinal tubules treated with potassium oxalate. Previous studies also showed dentin treated with potassium oxalate produced crystals in size similar to the dentinal tubule, some crystals in the intertubular area of the exposed dentin and many crystals in the dentinal tubules (Greenhill and Pashley, 1981; Pashley et al, 2001; Paes Leme et al, 2004). However, potassium oxalate has a relatively short-term effect, since calcium oxalate is dissolved by saliva (Suge et al, 1995).

Cow’s milk is a common part of human diet. Although milk contains about 4.8 gm of lactose per 100 gm of milk, the high concentration of calcium and phosphorus in the milk helps prevent
dissolution of the enamel, while casein and other proteins are protective against dental caries (Aimutis, 2004; Harper et al, 1987). The anticariogenic effect of cow’s milk may be due to the high concentration of phosphates, calcium and caseins having a major effect on its buffering capacity. The buffering capacity of milk is due to its ability to withstand a drop in pH, even in the presence of lactic acid, produced by bacteria (Lutchman et al, 2006; Al-Dabbas et al, 2011). Syed and Chadwick (2009) found the addition of 6.25 ml milk to 25 ml of a low-pH carbonated beverage can reduce tooth erosions by 50%. However, our study is the first to demonstrate cow’s milk reduces hydraulic conductance similar to desensitizing dentifrices.

Our study evaluated hydraulic conductance using fluid filtration, allowing us to use the same specimen repeatedly (Kijsamanmith et al, 2016). Studying hydraulic conductance is important because fluid flow through the dentin is related to dental pain (Charoenlarp et al, 2007; Vongsavan and Matthews, 2007).

In conclusion, milk and desensitizing dentifrices had similar abilities to significantly reduce the hydraulic conductance of human dentin in extracted teeth. Therefore, cow’s milk may provide an inexpensive easily available alternative to treat dentin hypersensitivity.

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