Journal of Dentistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Polyols and remineralisation of enamel subsurface lesions

Peiyan Shen, Glenn D. Walker, Yi Yuan, Coralie Reynolds, Eric C. Reynolds*

Oral Health Cooperative Research Centre, Melbourne Dental School, Bio21 Institute, The University of Melbourne, Melbourne, Victoria, Australia

ARTICLE INFO

Keywords:
Remineralisation
Chewing gum
Enamel caries
Saliva

ABSTRACT

Sugar-free chewing gum containing polyols has been demonstrated to reduce caries experience in randomised controlled clinical trials. A range of polyols (mannitol, sorbitol, xylitol and maltitol) can be found in sugar-free gums and it has been claimed that they can facilitate calcium uptake into enamel subsurface lesions promoting remineralisation.

Objectives: The aim of this study was to compare the effect of polyols on remineralisation of enamel subsurface lesions in vitro by artificial saliva (AS) and by AS containing the salivary biomimetic casein phosphopeptide amorphous calcium phosphate (CPP-ACP).

Methods: The polyols (12.6% w/v) and CPP-ACP (0.376% w/v) were used at physiologically relevant concentrations approximating those released into saliva during chewing a CPP-ACP/polyol chewing gum. Enamel subsurface lesions were exposed to one of the polyols (xylitol, sorbitol, maltitol, mannitol) in AS or AS containing CPP-ACP for 7 days at 37 °C with a change of solution each day. Remineralisation of the enamel subsurface lesions was measured by transverse microradiography.

Results: A statistical test for equivalence showed there was no difference in remineralisation between the AS solutions with or without any of the polyols. The AS + CPP-ACP solution substantially promoted remineralisation over AS alone independently of any polyol added.

Conclusion: This controlled *in vitro* study showed that polyols at physiologically relevant concentrations did not promote remineralisation of enamel subsurface lesions by facilitating calcium uptake into the lesion.

1. Introduction

Sugar-free chewing gums containing polyols have been demonstrated to reduce caries experience when compared with no gum chewing in randomised clinical trials [1–4]. The anticariogenic effect of chewing the sugar-free gum has been attributed to the stimulation of saliva [4,5]. Recently the anticariogenic efficacy of sugar-free gum has been enhanced by the addition of the salivary biomimetic casein phosphopeptide amorphous calcium phosphate (CPP-ACP) which significantly increases the buffering and remineralisation capacity of saliva by providing bioavailable calcium and phosphate ions stabilised by the CPP [6–16]. In a randomised controlled clinical trial a sugar-free chewing containing CPP-ACP with a sorbitol-mannitol (polyol) blend was significantly better than a control chewing gum containing only the polyols in slowing the progression of dental caries and enhancing regression of early lesions in children [10]. Enhanced remineralisation of enamel demineralised lesions and an increase in salivary calcium levels by sugar-free chewing gum containing CPP-ACP have been demonstrated in a number of other clinical studies [6–9,11,13–15,17,18].

Sugar-free chewing gum with or without CPP-ACP may be formulated using different combinations of sugar alcohols (polyols) such as xylitol, sorbitol, mannitol and maltitol [5]. The polyols act as both binding and sweetening agents in sugar-free gums and are all considered non-cariogenic as they are poorly fermented by oral bacteria [5,19]. The most common polyols used in sugar-free gums are xylitol and sorbitol although more recently blends of polyols are being used to provide certain taste characteristics [2,5,9,20].

Makinen and Soderling [21] have suggested that sorbitol and xylitol at very high concentrations in a saturated calcium sulphate solution form Ca^{2+}-polyol complexes through the formation of *cis-cis*-triol coordination complexes. Based on these findings the authors proposed that these polyols may influence calcium bioavailability in saliva and thereby may directly promote remineralisation of enamel subsurface lesions. Similarly, in an *in vitro* enamel lesion remineralisation study Miake et al. [22] suggested that a remineralisation solution containing 20% w/w xylitol produced less remineralisation in the outer layers of the lesion but greater remineralisation in the deeper layers compared with the solution without xylitol. The authors proposed that xylitol could influence remineralisation of deeper layers of demineralised enamel by facilitating Ca^{2+} movement into the lesion.

* Corresponding author.
E-mail addresses: k.fletcher@unimelb.edu.au, e.reynolds@unimelb.edu.au (E.C. Reynolds).

http://dx.doi.org/10.1016/j.jdent.2017.08.008
Received 22 June 2017; Received in revised form 10 August 2017; Accepted 16 August 2017

These results taken together suggest that the use of different polyols may effect the amount or pattern of remineralisation of enamel subsurface lesions by saliva or saliva/CPP-ACP. However, data from clinical studies suggested that chewing sugar-free gum containing CPP-ACP with either xylitol or sorbitol resulted in comparable remineralisation of enamel subsurface lesions [13], suggesting that, at physiologically relevant salivary concentrations of the polyols, no measurable difference in remineralisation could be detected. These inconsistent findings suggest further research is required to clarify the direct effect of polyols on enamel subsurface remineralisation. To date, no study has directly compared the remineralisation efficacy of saliva or saliva/CPP-ACP in the presence of the four commonly used polyols (xylitol, mannitol, sorbitol or maltitol) at physiologically relevant concentrations released by normal use of commercially available sugar-free chewing gum containing high levels of the polyols. Therefore, the hypothesis to be tested in this current in vitro study was that artificial saliva (AS), or AS/CPP-ACP, with and without xylitol, sorbitol, maltitol or mannitol at physiologically relevant concentrations were statistically equivalent with respect to their ability to remineralise enamel subsurface lesions.

2. Materials and methods

2.1. Enamel subsurface lesion preparation

Extracted human third molars were obtained from the Melbourne Dental School, The University of Melbourne after informed patient consent. The study was approved by the University of Melbourne's Human Research Ethics Committee (number 1136929). The teeth were first washed thoroughly in distilled deionised water (DDW) then sterilised with 4.1 kGy of gamma radiation. After sterilisation, any soft tissues were removed from the teeth and sound relatively planar buccal and lingual surfaces free of cracking, staining and fluorosis (as viewed under a dissecting microscope) were selected. The outer enamel surfaces were polished wet to a mirror finish using Soflex™ discs on a slow speed contra-angle dental handpiece. Each polished surface was cut from the tooth as an approximately 8 x 4 mm block, using a water-cooled diamond blade saw and the whole block was then covered with acid-resistant nail varnish except for two (occlusal and gingival) mesiodistal windows (approximately 1 x 8 mm each) separated from each other by 1 mm. The blocks were treated to create lesions in the enamel windows by suspending each block in 40 mL of unagitated demineralisation buffer, consisting of 80 mL/L Noverite K-702 polyacrylate solution (Lubrizol Corporation, Wickliffe, OH), 500 mg/L hydroxyapatite (Bio-Gel® HTP, Bio Rad Laboratories, Richmond CL), and 0.1 mol/L lactic acid (Ajax Chemicals, Auburn NSW) pH 4.8, for 4 days at 37 °C [23]. A change of solution was made after two days at which time the blocks were removed from the solution, rinsed thrice with DDW, blotted dry and placed into fresh demineralisation buffer. The blocks were similarly rinsed with DDW and dried after four days of demineralisation. This demineralisation procedure produces consistent subsurface lesions of 100 µm depths with intact surface layers, as evaluated by contact microradiography of sections of the lesions. After demineralisation, the enamel blocks were cut perpendicular to the windows into two 4 × 4 mm half-blocks and the cut surface of each block was covered with nail varnish. One of the half-blocks was retained as the demineralisation control and stored in a labeled 1.5 mL microcentrifuge tube together with a drop of DDW, thereby creating a humidified environment. The other half-block (test half-block) was used for remineralisation (see below).

2.2. Remineralisation protocol

A total of ten solutions were tested. One solution contained only artificial saliva (AS). AS consisted of 50 mM NaCl, 0.5 mM $CaCl_2$ and 0.5 mM Na_2HPO_4/NaH_2PO_4 pH 7.0. Four AS solutions contained 12.6% (w/v) of one of the following four polyols: xylitol, sorbitol, maltitol, or mannitol. This concentration of polyol was used as it is the maximum concentration attained in saliva while chewing with commercially-available gum (two pieces of gum) containing these compounds at the upper end of the normal range 0.5–1.0 g per piece of gum. The concentration was determined as the maximum concentration achieved in saliva while chewing two pieces of a high dose xylitol gum using stimulated saliva flow rates and release kinetics data [24,25]. Five AS solutions contained 0.376% (w/v) CPP-ACP and four of these also contained 12.6% (w/v) of one of the following four polyols: xylitol; sorbitol; maltitol; or mannitol.

Each test half enamel block was placed into 5 mL of one of the ten AS solutions and incubated at 37 °C for seven days. Each day the 5 mL AS solution was replaced with fresh 5 mL AS solution. Six half-blocks each containing two demineralised subsurface lesions were used for each of the ten AS remineralisation solutions.

2.3. Sectioning and transverse microradiography

Each demineralised control enamel half-block and remineralised test enamel half-block were rinsed in ethanol to remove the nail varnish and washed thoroughly in DDW. Each test half-block was paired with its corresponding control half-block, coded (blinded) and placed into freshly poured transparent cold curing methacrylate resin with the lesion windows parallel. The resin vial was marked at the top corner to identify the test and control half-blocks and the resin was allowed to set at room temperature overnight. Sections approximately 200 µm thick was cut from embedded blocks perpendicular to the lesion surface through the midline of both half-lesions using an internal annulus saw microtome. The sections were lapped down to 95 ± 5 µm using a RotoPol/RotoForce lapping instrument with 1200 and 2400 grit lapping paper. The lapped sections were removed from the lapping instrument with absolute ethanol and rinsed in deionised water, blotted dry and stored on soft tissue between glass slides. Each section, which contained the remineralised lesions and the demineralised control lesions from the same enamel block, was radiographed along with an aluminium stepwedge of 37.5 x 7 µm thick increments using Microchrome High Resolution glass plates (1 × 3 x 0.06 in., Microchrome, USA) and nickel filtered copper Ka radiation [26] at 20 kV, 30 mA for 8 min using a microradiography unit (XMR, Diffraction Technology Pty Ltd) with a PANalytical glass XRD fine focus tube with a copper target (Spectris Australia Pty Ltd) powered by a DF3 generator (Spellman High Voltage Electronics Corporation). Each glass plate was developed in Microchrome Developer D5 for 5 min, placed into glacial acetic acid stop bath for 30 s and then fixed in Microchrome Fixer F4 for 5 min.

2.4. Microdensitometry

Radiographic images of the lesions were viewed via transmitted light through a microscope. The images were acquired by a digital camera and analysed using imaging software Image Pro Plus Version 7.0. Images of the lesions and the neighboring areas of sound enamel were scanned using the programs line luminance function that gave readings in gray values. An area free of artifacts or cracks was selected for analysis. Each scan comprised 200 readings taken from the tooth surface through the lesion to sound enamel. The aluminum stepwedge image on each slide was scanned and the averaged step gray value readings were plotted against aluminum thickness. The readings of the tooth section image were within the linear portion of the stepwedge curve and linear regression was used to convert the gray value data into values of equivalent thickness of aluminum. The section thickness was measured and the % volume mineral (% vol min) data computed using the equation of Angmar [27] and the linear absorption coefficients of aluminum, organic matter plus water and apatitic mineral (131.5, 11.3 and 260.5 respectively). The image of the median strip of sound enamel between the two lesions was scanned six times and averaged to give a