Journal of Dentistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.intl.elsevierhealth.com/journals/jden

The effect of various model parameters on enamel caries lesions in a dose–response model in situ

H. Meyer-Lueckel [a], R.J. Wierichs [a,*], B. Gninka [b], P. Heldmann [b], C.E. Dörfer [b], S. Paris [c]

[a] Department of Operative Dentistry, Periodontology and Preventive Dentistry, RWTH Aachen University, Pauwelsstr. 30, 52074, Aachen, Germany
[b] Clinic for Operative Dentistry and Periodontology, School of Dental Medicine, Christian-Albrechts-Universitätzu Kiel, Arnold-Heller-Str. 3, 24105, Kiel, Germany
[c] Department of Operative and Preventive Dentistry, Charité–Universitätsmedizin Berlin, Assmannshauser Str. 4-6, 14197 Berlin

ARTICLE INFO

Article history:
Received 9 May 2015
Received in revised form 21 July 2015
Accepted 8 August 2015

Keywords:
Fluoride dose-response
In situ
Demineralization
Chlorhexidine
CHX
Lacalutaktiv

ABSTRACT

Objectives: The aim of this exploratory double-blinded, randomized, cross-over, in situ study was to compare the effects of various model parameters ('intervention', 'brushing', 'position') on enamel caries lesions in a dose-response model.
Methods: In each of four experimental legs of four weeks 16 participants wore intraoral mandibular appliances with four 'plaque-retaining' and four 'easily cleanable' positioned pre-demineralized bovine enamel specimens in the vestibular flanges mimicking proximal and buccal surfaces, respectively ($n = 512$). The four randomly allocated interventions (either application only or brushing) included the following dentifrices: AlF_3 1360 ppmF^- + chlorhexidine 0.05% (Lacalut aktiv, LA1360), NaF 1,450ppmF^-(Blend-a-Med ProExpert), NaF 500 ppm F^- and 0 ppm F^- as negative control (NC) (both experimental, based on Blend-a-Med ProExpert).
Results: Differences in integrated mineral loss ($\Delta\Delta Z$) and lesion depth (ΔLD) were calculated between values before and after the in situ period using transversal microradiography. Significant differences for $\Delta\Delta Z$ [adjusted mean (95% CI))] were found between NC, NaF500 and LA1360for both 'plaque-retaining' [−1830 (−2371;1289); −986 (−1530;442); −2 (−548;544) vol% × μm] as well as 'easily cleanable' specimens [−399 (−682; −116); −391 (−672; −110); −16 (−302;270) vol% × μm]. Values for NaF1450 revealed a similar dose-response as LA1360.Values for LA1360 and NaF1450 did not differ significantly ($p > 0.05$; ANCOVA).
Conclusion/Clinical Significance: The design of the present in situ study was able to reveal a fluoride dose-response to hamper further demineralization of enamel specimens for 'easily cleanable' and 'plaque-retaining' sites being brushed or not. Particularly 'plaque-retaining' sites seem to be recommendable for measuring potential anticaries efficacy in situ.

1. Introduction

For many years the effects of fluorides on caries incidence and prevalence have been studied and intensive research has been conducted to determine the benefits, safety and cost-effectiveness of various means of fluoride delivery [1]. Since clinical trials are rather demanding with respect to costs and study length, in situ studies, facilitating the control of experimental variables and shortening the duration of the study, enable the analysis of the caries process under complex, physiological conditions in the oral environment, while using in vitro sensitive state of the art analytical techniques [2,3]. For fluoride dentifrices respective models should be capable to demonstrate a significant fluoride dose-response [2–4].

Although several previous in situ models have shown that pre-demineralized enamel lesions remineralize after a few weeks, only a few of them demonstrated the required dose-response. For example a fluoride dose-response could be shown for dentifrices containing either 0, 250 or 1100 ppm fluoride (as NaF) in pre-demineralized human enamel specimens, situated in 'proximal' (plaque-retaining) position in partial dentures. Brushing was performed three times daily over a period of 28 days [5]. Another study revealed a fluoride dose-response after the same period, when brushing pre-demineralized human enamel specimens twice daily with dentifrices containing either 0, 1000, or 2500 ppm fluoride as sodium monofluorophosphate. In this study

* Corresponding author. Tel.: +49 241 80 88110; fax: +49 241 80 82468.
E-mail address: rwierichs@ukaachen.de (R.J. Wierichs).

http://dx.doi.org/10.1016/j.jdent.2015.08.003
0300-5712/

lingual mandibular appliances were used to simulate 'proximal' position [6].

So far these in situ models on toothpastes only revealed a fluoride dose response for one 'setting' (pre-demineralized human specimens in 'plaque-retaining' sites being brushed [5,6]. None of them demonstrated a fluoride dose-response for a second 'setting' as for non-brushed 'plaque-retaining' or 'easily cleanable' specimens (being brushed or not) simultaneously, although a more distinct inhibition of demineralization and a more pronounced remineralization could be revealed for 'easily cleanable' specimens compared with plaque-retaining specimens [7,8]. Moreover, both parameters, brushing or not as well as position of the specimens in either 'plaque-retaining' or 'easily cleanable' sites have not been studied simultaneously as well.

The ingredients of a dentifrice may vary widely depending on the oral health benefits it intends to provide (i.e. antimicrobial, anticaries, whitening properties). Interestingly, the main type of fluoride compounds, namely sodium monofluorophosphate, stannous fluoride, sodium fluoride and amine fluoride, might not influence the magnitude of the treatment effect [9]. In contrast, other reviews reported a significantly greater reduction in caries incidence for sodium fluoride compared with sodium monofluorophosphate containing toothpaste [10,11]. Besides these fluoride compounds, aluminum fluoride (AlF_3), a soluble salt of a polyvalent metal, could be of relevance as well. While providing as much fluoride as other fluoride compounds (e.g. NaF) it combines the remineralizing properties of fluoride with the antimicrobial properties of the aluminum [12]. Several studies demonstrated a synergistic cariostatic effect of aluminum and fluoride [12–14], since the positively charged aluminum may be incorporated in the enamel surface substituting calcium ions in hydroxyapatite and leading to a more stable complex than $CaHPO_4$. However, so far only three clinical studies investigated AlF_3. The antimicrobial effect was analyzed by using surrogate outcomes as digital plaque image analysis [15,16] or gingival index, plaque index, staining and calculus formation [17]. Remineralizing potential of an AlF_3-containing dentifrice has not yet been analyzed in an in situ or clinical study.

Thus, the aim of the present study was to evaluate the suitability of an in situ model by using brushed and non-brushed enamel specimens in either 'easily cleanable' or 'plaque-retaining' positions to reveal a dose-response of fluoride toothpaste. The products chosen for validating this model were dentifrices containing either 0, 500 or 1450 ppm fluoride (as NaF).

The secondary aim of the study was to evaluate if any of the various model parameters could differentiate the remineralizing potential of a 1360 ppm F^- (as aluminum fluoride)/chlorhexidine (AlF_3/CHX) dentifrice and a 1450 ppm F^- (as NaF) dentifrice. We hypothesized that both dentifrices containing regular fluoride concentrations inhibit significantly more demineralization compared to fluoride-free dentifrice.

2. Materials & methods

The study design was an exploratory double-blinded, randomized, cross-over, in situ trial with four treatment legs. Ethical approval was given by the local institutional review board (Christian-Albrechts-Universitätzu Kiel; No. A139/10).

The number of participants was calculated on the basis of previously performed in situ studies [18–20]. The α-error was set at 5%. Considering the differences between the 1450 ppm fluoride and 0 ppm fluoride for 'plaque-retaining' position and 'easily cleanable' position, the statistical power calculated was 99% (mean difference of 1000 (SD: 600)) and 99% (mean difference of 250 (SD: 200)), respectively. Dropout rate was assumed not to exceed 20%.

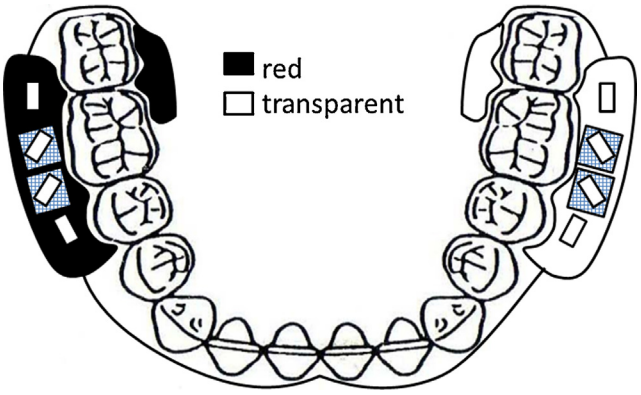

Fig. 1. Design of the intraoral mandibular appliances.
The acrylic resin of one side of the vestibular flanges appeared white/transparent, indicating that only this flange had to be brushed. Two specimens in the middle of each flange represented 'plaque-retaining' the other two 'easily cleanable' conditions.

Approximately 20 subjects should have been enrolled into the study for expected completion of at least 16.

All participants gave their written informed consent. They were all in good general health with no signs of active caries or periodontal disease. Exclusion criteria were: pregnancy, current participation in another study, institutionalized patients, periodontal disease, active caries lesions, age < 18, salivary flow rate < 0.7 ml/min, no written informed consent and incapability of contracting. After screening for general eligibility dental impressions of the lower jaw were taken and appliances were prepared [21]. The acrylic resin (Orthocryl; Dentaurum, Pforzheim, Germany) of one side of the vestibular flanges was transparent, indicating that only this flange had to be brushed (see below) (Fig. 1). In each of both flanges four pre-demineralized bovine enamel specimens were inserted [7,22]. Two 'easily cleanable' specimens (to mimic a 'buccal' surface) were fixed with sticky wax and were positioned flush with the acrylic surface. The other two 'plaque-retaining' specimens were inserted 1 mm below the acrylic under a plastic mesh (Perfect Splint®-System; Hager & Werken, Duisburg, Germany).

2.1. Randomization

After baseline examination a computerized random allocation sequence was generated by the study sponsor who coded the dentifrices with a subject number. The code was provided by the study sponsor (P&G, Mason, Ohio, USA) in sealed envelopes to be broken only in the case of an emergency.

2.2. Study design

The parameters under evaluation were:

- 'intervention' at four levels: application of [1] sodium fluoride (1,450 ppm F^-) containing dentifrice (Blend-a-Med ProExpert Rundumschutz: NaF1450), [2] sodium fluoride (500 ppm F^-) containing dentifrice (NaF500), [3] fluoride-free dentifrice (0 ppm F^-) (negative control, NC), (both experimental, based on Blend-a-Med ProExpert Rundumschutz) or [4] chlorhexidine digluconate (0.05%), aluminum lactate (0.8%) and aluminum fluoride (AlF_3) (1360 ppm F^-) containing dentifrice (Lacalut aktiv: LA1360);
- 'brushing' at two levels: yes (B) or no (NB) and
- 'position' of the specimen at two levels: 'easily cleanable' or 'plaque-retaining'