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Dose–response effect of fluoride dentifrice on remineralisation and further demineralisation of erosive lesions: A randomised *in situ* clinical study

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ABSTRACT

Objective: The objective was to evaluate the ability of fluoride in a conventional, non-specialised sodium fluoride–silica dentifrice to promote tooth remineralisation and enamel fluoride uptake (EFU), and assess the resistance of the newly formed mineral to attack by dietary acid, across the concentration range used in mass-market dentifrices.

Methods: Subjects wore a palatal appliance containing eight polished bovine enamel specimens, each including an early erosive lesion. In a randomised full-crossover sequence, 62 healthy subjects were treated with dentifrices containing four different fluoride concentrations: no fluoride; 250 ppm, 1150 ppm and 1426 ppm fluoride. At each treatment visit, under supervision, subjects brushed with 1.5 g dentifrice and rinsed once while wearing the appliance; the appliance was removed after a 4-h remineralisation period and effects on the enamel specimens determined. The primary efficacy variable was surface microhardness recovery (SMHR); others included EFU, relative erosion resistance (RER) and comparative erosion resistance.

Results: Highly significant linear and, with the exception of SMHR, quadratic dose–response relationships were observed between all efficacy variables and fluoride concentration. For SMHR, EFU and RER, values for the different fluoride concentrations were statistically resolved from one another, with the exception of the two highest fluoride concentrations. The degree of remineralisation and the acid resistance of enamel after treatment were closely related to EFU.

Conclusion: After a single brushing, conventional non-specialised sodium fluoride–silica dentifrices promoted remineralisation of early enamel lesions, and imparted increased acid-resistance to the enamel surface, in a dose-dependent manner at least up to 1500 ppm fluoride.

Clinical significance: Enamel erosive tissue loss is an increasing concern, associated with modern diets. This study demonstrated that sodium fluoride, in a conventional non-specialised dentifrice formulation, can promote repair of the earliest stages of enamel erosion after a single application, in a dose-dependent fashion across the fluoride concentration range used in mass-market dentifrices.

This study is registered in the GlaxoSmithKline Study Register (ID RH01299), available at: www.gsk-clinicalstudyregister.com/study/RH01299.

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1. Introduction

Exposure to erosive acids can result in demineralisation of enamel surfaces, increasing their susceptibility to abrasive forces and leading to erosive tooth wear.[1] Enamel topical fluoride application, such as rinsing with fluoride-containing solutions or using a fluoride-containing dentifrice, promotes remineralisation in the presence of salivary calcium and phosphate ions and forms a more acid-resistant fluoride-rich mineral layer on the enamel surface, directly reducing subsequent demineralisation. Both processes can reduce tooth wear.[2–6]

Recent *in situ* clinical studies have demonstrated significant dose-related remineralisation effects on early enamel erosive lesions, using specialised anti-sensitivity toothpastes containing 0–1150 parts per million (ppm) fluoride as sodium fluoride, in a base containing 5% potassium nitrate,[7,8] solely non-ionic surfactant, and a relatively low level of abrasive. However, no *in situ* clinical information is available on the effects of 'regular' dentifrices, i.e. daily-use conventional sodium fluoride dentifrices, which do not contain potassium salts, but do contain anionic surfactants and higher levels of silica abrasive, on remineralisation of erosive lesions. Nor are there dose–response data for any sodium fluoride formulations extending up to 1500 ppm fluoride, which is the most frequently used adult level globally.

The process of dental erosion by acidic foods and/or drinks in the diet is complex and influenced by chemical, biological and behavioural factors[3,4,9] that complicate the development of reproducible *in vitro* models. *In situ* appliances allow for the study of the enamel demineralisation and remineralisation process under well-controlled conditions in the oral cavity.[10] Such models allow the erosion repair process to be explored using sensitive analytical techniques without affecting the subjects' natural dentition, with much-reduced sample size relative to conventional caries clinical studies.[11]

The aim of the present study was to investigate the dose–response to fluoride concentration in the ability of a conventional, non-specialised daily-use sodium fluoride–silica dentifrice formulation to remineralise eroded enamel. The study further aimed to determine if the remineralised enamel so formed was more acid-resistant than the native enamel. The range of fluoride concentrations used spanned the range used globally, and specifically included the two most commonly used mass-market fluoride concentrations (1150 ppm fluoride and 1426 ppm fluoride, the latter used in countries where the limit is 1500 ppm fluoride).

2. Materials and methods

2.1. Study design

This was a single-centre, four-way, randomised, blinded (subject and investigator/examiner) crossover study involving four dentifrice treatments comparing the ability of dentifrices containing 250, 1150 and 1426 ppm fluoride as sodium fluoride in an experimental silica base, with a reference dentifrice with no added fluoride in the same silica base, to remineralise early erosive lesions in bovine enamel. All products were manufactured by GlaxoSmithKline Consumer Healthcare (GSKCH), UK.

The study population was composed of healthy adults (aged 18–65 years) with intact maxillary and mandibular dental arches and an unstimulated/stimulated salivary flow rate of $\geq$0.2 mL/min and 0.8 mL/min, respectively. Exclusion criteria included any medical history that could prevent completion of the study (e.g. diabetes); the presence of active caries (unless repaired prior to study) or moderate or severe periodontal disease that could compromise the study or the health of the subject; subjects wearing any oral appliance or orthodontia; and subjects taking a medication that could interfere significantly with saliva flow.

The study was conducted at the Oral Health Research Institute of the Indiana University School of Dentistry, Indianapolis, USA in compliance with the Declaration of Helsinki and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH); the protocol was approved by the Indiana University Institutional Review Board (IU IRB # 1201007834). All subjects completed an informed consent process, concluding with each providing a signature to acknowledge their understanding.

2.2. Sample size

Sample size was based on calculations from a study of essentially similar design, in which the standard deviation for the difference between a test dentifrice and a reference dentifrice was 10.2 for % surface microhardness recovery (SMHR) and 16.0 for % relative erosion resistance (RER) [GSK, unpublished data]. A sample size of 50 subjects completing a crossover study was calculated to have 80% power to detect differences of 4.1 for %SMHR and 6.5 for %RER between two products, assuming two-sided paired tests at a 5% significance level. For a typical expected response for a fluoride dentifrice of 30% remineralisation, a 4.1% increase in SMHR (i.e. to 34.1%) represents a relative increase in remineralisation of 13.7%.

A total of 73 subjects were screened and 62 subjects were randomised to allow for up to a 20% dropout rate, so that at least 50 subjects would complete the study. Sixty subjects completed all four treatment periods.

2.3. Randomisation

The randomisation schedule was provided by the Biostatistics Department, GSKCH and all parties were blinded. Each subject screened for study participation was assigned a unique screening number in ascending numerical order as the subject was determined to be fully eligible at Visit 3. Subjects who met all inclusion criteria were randomised according to the randomisation schedule. Subjects who withdrew from the study post-randomisation were not replaced.

2.4. Clinical procedures

The study used the *in situ* remineralisation model developed by Zero et al.[8] A tailored oral appliance used eight bovine enamel specimens mounted on two plastic holders as the hard

tissue substrate. The bovine enamel specimens were exposed for 25 min to an *in vitro* erosive challenge with grapefruit juice after baseline assessments and prior to the *in situ* remineralisation test. The full procedure has been described previously by Zero et al.[8,12]

The study duration for each subject was approximately 10 weeks and included the screening visit, the appliance try-in visit and four treatment visits, the latter at approximately two-week intervals.

2.4.1. *Screening*

At the screening visit (Visit 1), eligibility criteria, medical history, concomitant medications, stimulated/unstimulated salivary flow-rates, oral soft tissue (OST) and oral hard tissue (OHT) examinations were performed. In addition, subjects had an impression taken of their maxillary and mandibular arches (if an appliance was not already on-site) for the purpose of constructing the *in situ* palatal appliance.[8,13]

2.4.2. *Treatment phase*

At each treatment visit, continued eligibility criteria, medical history and concomitant medications were updated, and the subject received an OST exam. Subjects inserted their palatal appliance into their mouth for a 5-min equilibration period. Subjects then brushed the buccal surfaces of their teeth with 1.5 g of test toothpaste for 25 s and swished the slurry around their mouths for a further 1 min to allow direct contact with the enamel specimens. After expectorating the slurry, the subjects rinsed their mouths with 15 mL of tap water for 10 s before expectorating again. During the post-treatment periods, subjects remained on the site and wore their palatal appliance. Participants were instructed to refrain from talking for the first hour and not to eat or drink during the 4-h test period, although participants were allowed to drink water after 2 h upon removal of the appliance. The appliance was disinfected after each treatment period.

2.5. *Treatments*

Subjects received the following study treatments in a predetermined random order according to the randomisation schedule:

1. No added fluoride in a silica base.
2. 250 ppm fluoride as sodium fluoride in a silica base.
3. 1150 ppm fluoride as sodium fluoride in a silica base.
4. 1426 ppm fluoride as sodium fluoride in a silica base.

The base was identical in each case, containing just less than 14% silica abrasive and 1.5% of the anionic surfactant sodium lauryl sulfate, bar minor adjustments in water content to allow for the different fluoride levels.

2.6. *Outcomes and assessments*

The primary endpoint was the change in mineralisation status of the *in situ* eroded enamel across the fluoride ion concentration range 0 ppm to 1426 ppm, measured by change in SMH. Secondary endpoints were the enamel resistance to a post-remineralisation erosive challenge, assessed using the RER measure, and the level of fluoride delivered to eroded enamel *in situ*, as measured by enamel fluoride uptake (EFU). An additional exploratory endpoint was enamel resistance to a post-remineralisation erosive challenge, assessed using the comparative erosion resistance measure (CER). All paired comparisons between study formulations were made with regard to the above endpoints.

Assessments of tolerability were made with respect to OST abnormalities and adverse events (AEs) reported by subjects following use of the first study treatment regimens.

2.6.1. *Efficacy assessments*

2.6.1.1. Indentations. Each of the indentation values of enamel specimens at baseline (B), after first erosive challenge (E1), after remineralisation (R), and after second erosive challenge (E2) were the average values of the indentations made (maximum of five). For all assessments (SMHR, EFU, RER, CER), if a subject was missing an enamel specimen, the mean was computed over the available enamel specimens.

2.6.1.2. Remineralisation of eroded enamel in situ (SMHR). The SMH was determined prior to the *in vitro* erosive challenge (baseline), after the *in vitro* erosive challenge, after *in situ* remineralisation, and after a second *in vitro* erosive challenge.

SMH was used to assess changes in mineralisation status of enamel specimens using a Wilson 2100 Hardness tester. SMH was determined by measuring the length of the indentations in enamel specimens (see Fig. 1). An increase in the indentation length compared with baseline indicates softening, while a decrease in the indentation length represents rehardening of the enamel surface. Percent SMHR was calculated from indentation values of enamel specimens at B, E1 and R based on the method of Gelhard et al.[14]:

$$\left[\frac{(E1-R)}{(E1-B)}\right] * 100.$$

The positioning of the indentations is shown in Fig. 1.

The mean %SMHR for each subject for each treatment was determined from the %SMHR calculated for each specimen averaged across the eight enamel specimens per subject. Therefore, a single observation per treatment for each subject was used in the statistical analyses.

2.6.1.3. Enamel resistance to a post-remineralisation erosive challenge (RER). Enamel specimens were exposed to a post-treatment erosion challenge to determine RER, which compared the indentation values of enamel specimens at B, E1 and E2. Percent RER was calculated by a formula, based on the method of Corpron[15]:

$$\left[\frac{(E1-E2)}{(E1-B)}\right] * 100.$$

The subject-wise %RER was determined by averaging across the eight enamel specimens per subject.

2.6.1.4. Enamel resistance to a post-remineralisation erosive challenge (CER). The CER was developed as part of the present study, as an exploratory variable to understand the effect of

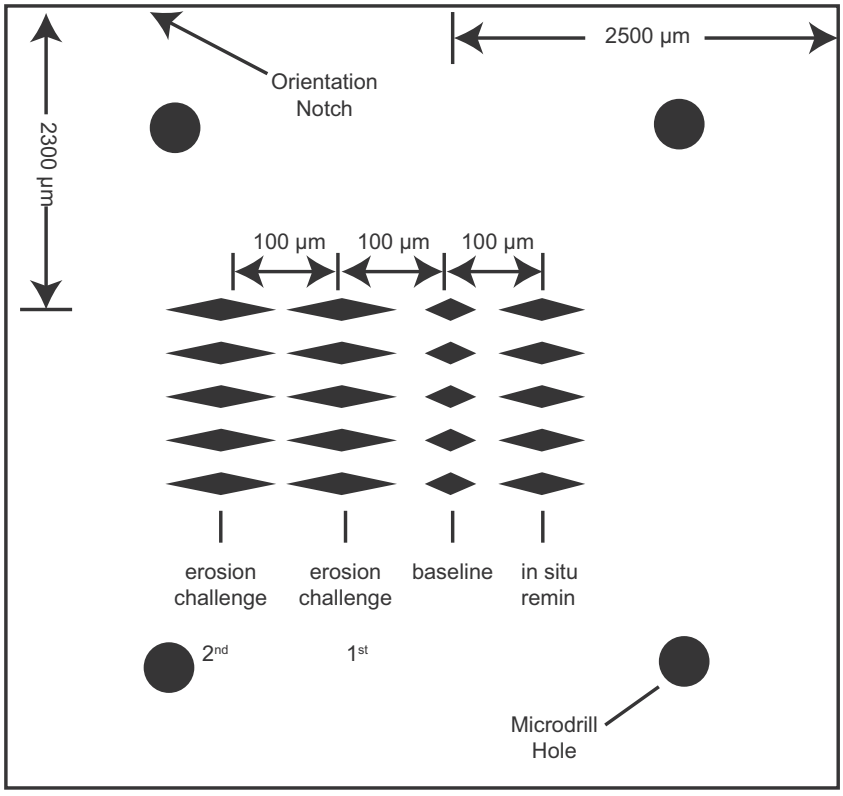

Fig. 1 – Bovine enamel specimen (5 mm by 5 mm) showing sites of the microdrill sample extractions and layout of indentations at baseline, after each *in vitro* erosion challenge and after the *in situ* remineralisation test. Five baseline indentations were made 100 micrometres (µm) apart. Four microdrill holes were made to a depth of ~200 µm through the entire lesion.

treatment on acid resistance of the enamel by comparing the demineralising effect of the post-treatment acid challenge with the demineralisation effect of the pre-treatment acid challenge. The measure is calculated as follows:

$$\%\text{CER} = \left[\frac{(\text{E2} - \text{R})}{(\text{E1} - \text{B})}\right] * 100.$$

2.6.1.5. *Fluoride delivered to eroded enamel in situ (EFU).* EFU was determined using the microdrill enamel biopsy technique of Sakkab et al.[16] The subject-wise EFU score was calculated by pooling four microdrill samples from each enamel specimen (see Fig. 1), and calculating the amount of fluoride uptake by enamel, based on the amount of fluoride divided by surface area of the enamel cores.

During analysis of EFU data, a high degree of variation was observed across the study. The main source of this variation was attributable to values calculated using a specific set of fluoride standards, which were found to be faulty. Approximately one-third of the data were affected. As a result, fluoride concentrations originally generated with faulty standards were recalculated using mean millivolt values obtained with accurate standards.

2.6.2. *Tolerability assessments*

2.6.2.1. *OST examination.* OST examinations were performed by the study dentist at each visit and comprised a visual examination of the oral cavity and perioral area; observations were classified as 'normal' or 'abnormal'. All abnormal changes noted after the screening visit, or which were present at the screening examination, but worsened during any of the treatment periods, were recorded as adverse events (AEs).

2.6.2.2. *AEs.* AEs were collected from the start of use of the washout dentifrice and until 5 days following last administration of the investigational dentifrice. AEs were assessed as to whether they were serious (e.g. were life-threatening or resulted in disability or hospitalisation), and whether they related to a study treatment (i.e. investigational, reference or wash-out product) or related to study participation (e.g. protocol-mandated procedures, invasive tests, or change in existing therapy).

2.7. *Statistical analysis*

Analysis of variance (ANOVA) was used to analyse SMHR, EFU, RER and CER. The ANOVA model included fixed factors for study period and treatment, and a random effect for subject. Mean treatment differences were calculated along with associated 95% confidence intervals (CIs) and *p*-values. All tests of hypotheses were two-sided at 5% significance level. Linear and quadratic contrasts were fitted in order to establish whether there was a dose–response relationship. All paired comparisons were made to compare the different dentifrice fluoride concentrations.

3. Results

3.1. Study timing, patient numbers and demographics

The first subject enrolled on 07 March 2012; the last subject completed treatment on 21 May 2012. A total of 73 participants were screened; of these, 62 were randomised and included in the intention-to-treat (ITT), safety and per protocol (PP) populations. The majority of the participants were white (59.7%) and the mean (SD) age was 36.7 (12.16) years. Slightly more females (56.5%) than males (43.5%) were included in the study (Table 1). A breakdown of disposition of subjects is presented in Fig. 2.

Table 1 – Demographic and baseline characteristics (safety/ITT population).

	Overall (N = 62)
Sex, n (%)	
Male	27 (43.5)
Female	35 (56.5)
Race, n (%)	
American Indian or Alaska native	1 (1.6)
Asian	6 (9.7)
Black or African American	17 (27.4)
Native Hawaiian or other Pacific Islander	0
White	37 (59.7)
Multiple	1 (1.6)
Ethnicity, n (%)	
Hispanic or Latino	6 (9.7)
Not Hispanic or Latino	56 (90.3)
Age, years	
Mean (SD)	36.7 (12.16)
Median (min–max)	34.5 (19–64)

ITT, intent to treat; max, maximum; min, minimum; *n*, number of participants; SD, standard deviation.

3.2. Efficacy results

3.2.1. SMHR

The progression of SMH values for the different treatments through the study is shown in Fig. 3. These values support the SMHR, RER and CER calculations. Mean SMHR increased with increasing dentifrice fluoride concentration (Table 2). A highly significant linear dose–response relationship was shown between SMHR and fluoride concentration (Fig. 4a). The quadratic dose–response relationship between dentifrice fluoride concentration and SMHR did not reach statistical significance. With the exception of the two highest fluoride concentrations, SMHR values for all fluoride concentrations were statistically significantly different from each other (Table 2).

3.2.2. RER

Mean RER increased with increasing dentifrice fluoride concentration (Table 2). Highly significant linear and quadratic dose–response relationships were observed between RER and fluoride concentration (Fig. 4b). With the exception of the two highest fluoride concentrations, RER values for all fluoride

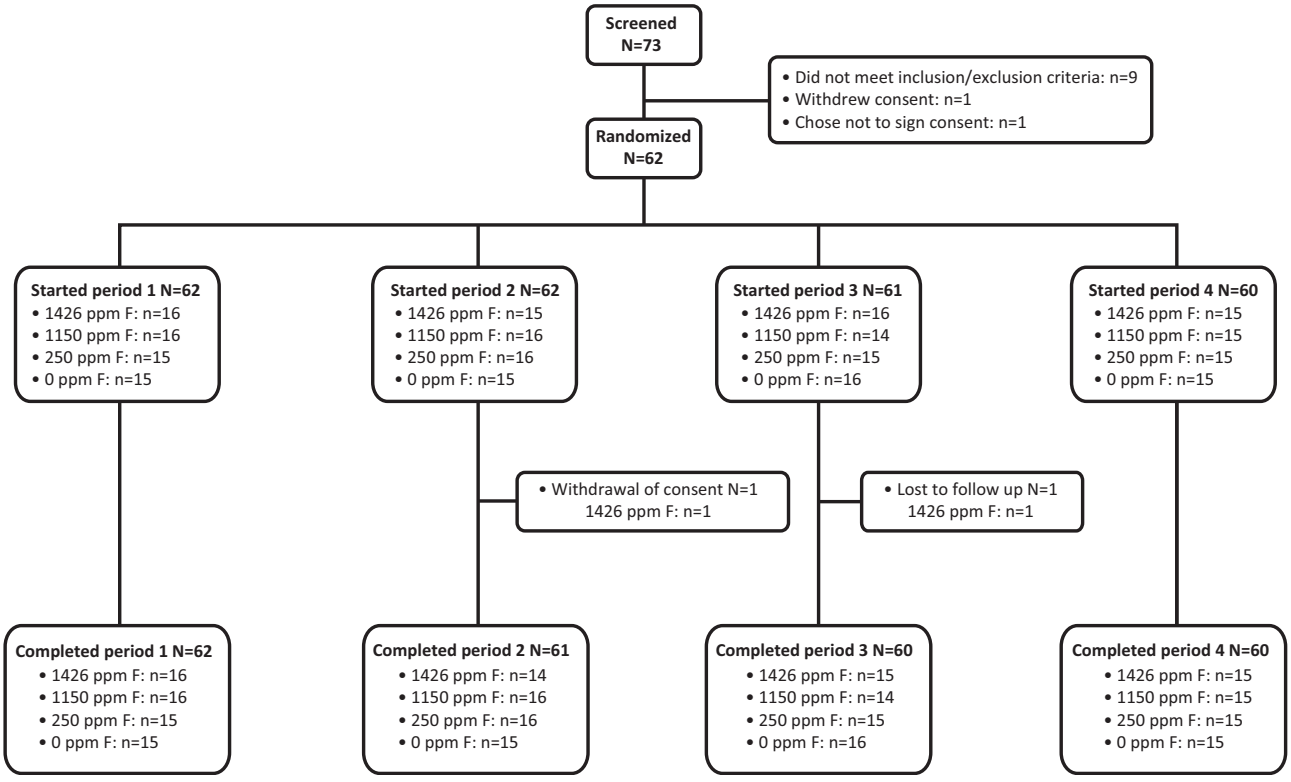

Fig. 2 – Participant flow diagram: subject disposition by treatment period. F, fluoride and ppm, parts per million.

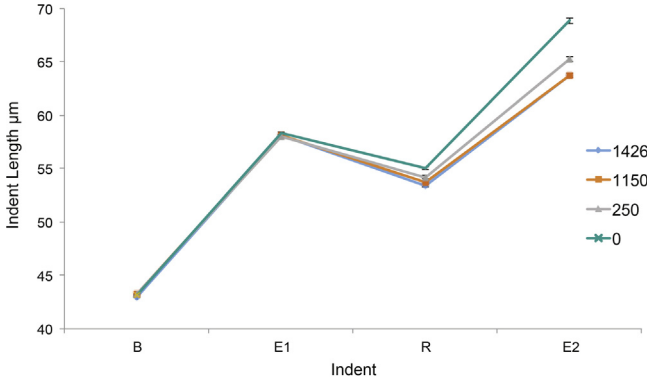

Fig. 3 – Progression of SMH values for the different treatments through the experimental stages. B, baseline; E1, first erosive challenge; R, *in situ* remineralisation; E2, second erosive challenge. Legend values are ppm fluoride in the dentifrice.

concentrations were statistically significantly different from each other (Table 2).

3.2.3. *EFU*

The EFU analysis using corrected data is shown in Table 2. Calculation of EFU values using three approaches (original unadjusted data; only data obtained with accurate standards; and data obtained with accurate standards together with data obtained for affected samples after recalculation with accurate standards) did not affect the rank order of EFU values for the different treatments. The only effect on discrimination between treatments was that in the original unadjusted data-set, the 0 ppm fluoride and 250 ppm fluoride were not statistically significantly different. However these two values became significantly different when using corrected data, as was also the case when the subset of data obtained with accurate standards was analysed. This result, therefore, indicates that the recalculation was effective in addressing the erroneous fluoride standards issue, so only the results from the data-set derived from the corrected EFU data are shown. Fig. 4c shows mean EFU values as a function of fluoride concentration based on the data-set using the recalculated EFU values. For this data-set, EFU increased with increasing dentifrice fluoride concentration, and highly significant linear and quadratic dose–response relationships were observed between EFU and fluoride concentration. With the exception of the two highest fluoride concentrations, EFU values for all fluoride concentrations were statistically significantly different from each other (Table 2).

3.2.4. *CER*

Mean CER values decreased with increasing dentifrice fluoride concentration (Table 2), indicating greater enamel acid resistance after the higher fluoride treatments. Highly significant linear and quadratic dose–response relationships were shown between CER and fluoride concentration (Fig. 4d). The CER values for the 0 ppm fluoride, 250 ppm fluoride and 1150 ppm fluoride dentifrice were statistically significantly different from all other treatments; however, the value for the 1426 ppm fluoride treatment was not significantly different from the values for either the 1150 ppm fluoride or the 250 ppm fluoride treatments.

3.3. *Safety results*

All treatments were well-tolerated in this study. A total of 14 treatment-emergent AEs were reported by 11 participants. Eight of the AEs were oral AEs, reported by six participants. All AEs were mild or moderate in intensity and none of the AEs led to withdrawal from the study. No serious AEs were reported. One AE, mouth ulceration observed in a participant receiving the 0 ppm fluoride formulation, was considered as being possibly related to treatment. The non-oral AEs consisted of single cases of cough; common cold; headache; sinus headache; muscular soreness, stomach ache. None of these was linked to study products, and none led to the subject's withdrawal from the study.

4. Discussion

The analysis used to monitor hardness, SMH, has been shown to provide a sensitive, reproducible way to detect changes in mineral content after *in situ* demineralisation or remineralisation of enamel.[7,10,12,13,17] SMH recovery in relatively superficial enamel lesions is generally accepted as representing remineralisation (and hence repair) of the lesion. Therefore, the positive relationship between SMHR and fluoride concentration indicates that fluoride in the experimental dentifrice formulation can promote repair of early erosive lesions in

Table 2 – Summary of analysis of SMHR, RER, EFU and CER (per protocol population).

Measure	Dentifrice fluoride content (ppm fluoride)				*p* value	
	1426 (*N* = 62)	1150 (*N* = 62)	250 (*N* = 62)	0 (*N* = 62)	Linear dose–response	Quadratic dose–response
%SMHR	30.9 (1.38)[a]	28.7 (1.38)[a]	25.3 (1.38)[b]	21.0 (1.39)[c]	<0.0001	0.3748
%RER	−38.8 (2.75)[a]	−39.7 (2.75)[a]	−50.4 (2.75)[b]	−71.2 (2.77)[c]	<0.0001	0.0002
EFU (µg F/cm^2)	3.13 (0.09)[a]	3.07 (0.09)[a]	2.09 (0.09)[b]	1.47 (0.09)[c]	<0.0001	0.0008
%CER	69.8 (2.66)[ab]	68.5 (2.66)[a]	75.7 (2.66)[b]	92.3 (2.68)[c]	<0.0001	0.0005

%SMHR, percentage surface microhardness recovery; EFU, enamel fluoride uptake (EFU includes all data, including a correction applied to the data where an incorrect standard had been used); %RER, percentage relative erosion resistance; %CER, percentage comparative erosion resistance. Values are adjusted means and within-subject SEs calculated from an analysis of variance (ANOVA) model, with treatment and period as factors, and subject as a random factor. Treatments with the same letter are not significantly different from each other (within row).

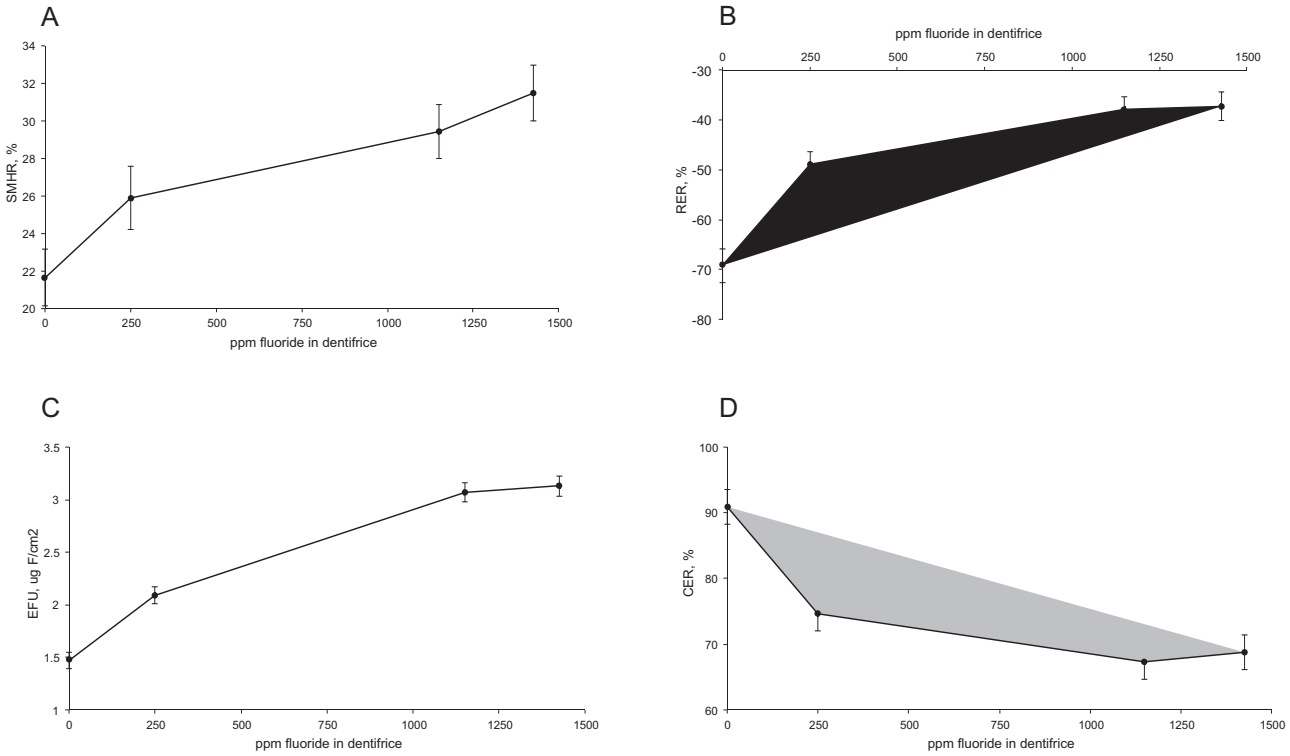

Fig. 4 – Dose–response relationship between dentifrice fluoride concentration and (a) SMHR; (b) RER; (c) EFU; and (d) CER. For each figure, means are adjusted means and error bars are within-subject standard errors from ANOVA model with treatment and period as factors, and subject as random factor.

enamel *in situ* after a single treatment. Specifically, the highly significant linear dose–response relationship between SMHR and dentifrice fluoride concentration across the range 0–1426 ppm fluoride achieved the primary objective of the study. This outcome is consistent with results obtained previously with specialised potassium nitrate-containing dentifrice formulations with fluoride levels up to 1150 ppm.[7]

All the variables investigated in this study – SMHR, EFU, RER and CER – showed a broadly similar relationship with fluoride concentration in the dentifrice. The CER and RER results indicate that the acid resistance of the surface, including new mineral formed during the post-brushing remineralisation period, increased with the concentration of fluoride in the dentifrice. In all the analyses, the 1426 ppm fluoride concentration did not offer a statistically significant advantage over the 1150 ppm concentration, which might suggest that in this clinical model the fluoride benefit was approaching saturation. However, these two fluoride concentrations are relatively close together, and a more highly powered study would be required to determine whether there was a measurable difference in performance between them. The better fit obtained to the dose–response SMHR data using a linear fit compared to a quadratic fit does suggest that the SMHR response is not yet reaching saturation at 1426 ppm fluoride. The study encourages investigation of the *in situ* effects of fluoride levels in dentifrices above 1500 ppm fluoride, to determine if they may provide enhanced protection against erosive challenge, as has been found for caries.[18,19]

For EFU, RER and CER, highly significant quadratic (as well as linear) relationships were observed, unlike for SMHR. Further work with specifically chosen fluoride concentrations is needed to clarify the linear-versus-hyperbolic nature of the dose–response relationships for these different variables.

The amount of fluoride taken up into the enamel during the remineralisation process was closely related to the efficacy variables measured: SMHR, RER and CER. This is consistent with fluoride's proposed mechanism of action in promoting remineralisation of early enamel lesions, and becoming itself adsorbed onto the lesion surface and incorporated into the new mineral formed, enhancing the surface acid resistance.[20–22]

RER has been extensively used to investigate whether material deposited during the remineralisation phase, after use of a test product, is more resistant to acid attack than the material deposited during the remineralisation phase after use of a reference product.[7,15] The RER calculation compares the difference between the SMH after initial demineralisation and after final demineralisation, with the difference between the SMH at baseline and after final demineralisation.[15] This means, as well as comparing the effect of the pre- and post-treatment acid challenges, the RER also includes the remineralisation effect of the treatment in the calculation. Hence, the RER may best be considered as an overall estimate of the effect on the enamel surface, i.e. combining the treatment effects on promoting remineralisation together with its effects on inhibiting demineralisation, into an estimate of the total treatment benefit.

The new experimental analysis, CER, was developed to focus exclusively on the ability of the treatments to impart a greater acid resistance to enamel. It achieves this by comparing directly the effect of the final demineralisation (i.e. the difference between SMH after remineralisation with SMH after post-treatment demineralisation) with the effect of the initial demineralisation (i.e. the difference between SMH at baseline and SMH after pre-treatment demineralisation). The degree of remineralisation due to treatment is not considered. While this approach is vulnerable to bias, if there is variation in the rate of demineralisation as a function of the depth of the lesion, this bias is expected to be very modest for very superficial lesions such as those examined in this study. Consistent with this expectation, for the fluoride-free treatment, the pre- and post-treatment demineralisation challenges produced a very similar change in enamel SMH, even though the enamel was considerably more demineralised at the start of the post-treatment challenge than it was at the start of the pre-treatment challenge (with resulting CER value of over 90%).

In this study, the clear relationship between fluoride concentration in the dentifrice and the new experimental analytical variable CER indicates that fluoride delivered by the dentifrice increases the acid-resistance of the treated enamel surface in a clearly dose-dependent manner. Direct comparison of the effect of the pre- and post-remineralisation acid challenges appears to give a useful estimate of acquired enamel acid resistance due to treatment that, if confirmed in further work, could be adopted as a standard measure of treatment-induced acid resistance in remineralisation studies.

5. Conclusions

This study showed that fluoride in this conventional, non-specialised sodium fluoride–silica dentifrice formulation provided protection against dietary acid attack. This protection was demonstrated by the dentifrice's ability to remineralise early erosive lesions in bovine enamel *in situ*, and to impart resistance of the remineralised lesions to subsequent erosive challenge. The formulation was progressively more effective as fluoride concentration increased across the range 0–1426 ppm, though there was evidence that a plateau was being approached in the test model used in this study.

Conflicts of interest

JE Creeth, ML Bosma, A Butler and RJM Lynch are employees of GSK Consumer Healthcare. DT Zero receives compensation from GSK Consumer Healthcare as a consultant. This study was funded by GSK Consumer healthcare. SA Kelly, EA Martinez-Mier, and A Hara report no conflicts of interest.

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All authors drafted the article or revised it critically for intellectual content and approved the final version to be submitted. Authors that conceived and designed aspects of the study: JEC, SAK, EAM-M, AH, MLB, RJML, DTZ; authors that designed and performed analysis of the study: AB; authors that led the site clinical team: SAK, AH.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdent.2015.03.008.

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