Original Paper

Caries Research

Caries Res 2013;47:355–363
DOI: 10.1159/000348594

Received: August 16, 2012
Accepted after revision: February 1, 2013
Published online: April 5, 2013

The Effect of Monoalkyl Phosphates and Fluoride on Dissolution of Hydroxyapatite, and Interactions with Saliva

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Key Words
Dental erosion · Dissolution · Fluoride · Hydroxyapatite · Monoalkyl phosphates · pH-stat

Abstract
The aims were to investigate the effect of monoalkyl phosphates (MAPs) and fluoride on dissolution rate of native and saliva-coated hydroxyapatite (HA). Fluoride at 300 mg/l (as NaF) inhibited dissolution of native HA by 12%, while potassium and sodium dodecyl phosphates (PDP, SDP), at 0.1% or higher, inhibited dissolution by 26–34%. MAPs, but not fluoride, also showed persistence of action. MAPs at 0.5% and fluoride at 300 mg/l were then tested separately against HA pre-treated with human saliva for 2 or 18 h. Agents were applied with brushing to half the specimens, and without brushing to the other half. In control (water-treated) specimens, pre-treatment of HA with human saliva reduced dissolution rate on average by 41% (2 h) and 63% (18 h). Brushing did not have a statistically significant effect on dissolution rate of saliva-coated specimens. In brushed specimens, fluoride significantly increased the inhibition due to 2- or 18-hour saliva pre-treatment. It is hypothesised that brushing partially removes the salivary film and allows KOH-soluble calcium fluoride formation at the surfaces of HA particles. Inhibition was reduced by PDP in 2-hour/non-brushed specimens and in 18-hour/brushed specimens. PDP did not affect dissolution rates in the remaining groups and SDP did not affect dissolution rate in any group. Possible reasons for these variable results are discussed. The experiments show that pre-treatment with saliva can significantly modify results of tests on potential anti-erosive agents and it is recommended that saliva pre-treatment should be a routine part of testing such agents.

In dental erosion tooth surfaces are partly dissolved by acids, which originate in some patients from regurgitated gastric contents but in most from acidic dietary products, including soft drinks. The resulting softening of the tooth surface leads to abnormally rapid tooth wear and, eventually, to significant loss of tooth tissue and impaired function [Lussi et al., 2011]. Dental erosion is a widespread phenomenon that affects both children and adults; some studies estimate that up to 50% of 2- to 5-year-olds and up to 100% of 9- to 17-year-olds exhibit erosive wear [Jaeggi and Lussi, 2006]. There is, therefore, considerable interest in the development of methods to inhibit dental erosion but prevention poses

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major problems because the erosive challenge is much more severe than that in dental caries. Thus, fluoride, which is well established in caries prevention, is much less effective against dental erosion. Conventional fluoride preparations such as toothpastes or mouth rinses have a limited effect on erosion [Wiegand and Attin, 2003; Magalhães et al., 2011] and significant inhibition requires either application in high concentrations and at high frequency [Ganss et al., 2004], or use of preparations containing titanium or stannous ions [Ganss et al., 2010; Wiegand et al., 2010] that are unsuitable for routine use because of their low pH and their propensity to stain teeth. One alternative approach is to reduce the erosive potential of individual food and drink products, by increasing the pH or adding component ions of hydroxyapatite (HA) or other surface-active compounds, but this approach is only suitable for a few foods and drinks. Another approach is to design oral healthcare products which may be applied at intervals to the teeth and provide protection over a prolonged period by modifying the tooth surfaces.

Some inorganic polyphosphates and polymers containing phosphate moieties bind particularly effectively to HA and enhance resistance to demineralisation [McGaughey and Stowell, 1977; Schaad et al., 1994; Barbour et al., 2005]. The present paper is concerned with a different class of phosphates: monoalkyl phosphates (MAPs). These are anionic surfactants comprising a hydrophilic phosphate head group esterified to a single hydrophobic hydrocarbon chain. MAPs with C_2, C_4 and C_6 tail lengths have been shown to adsorb to HA surfaces [Ishikawa et al., 1995; Tanaka et al., 1997a, b]. As these properties could modify the susceptibility of HA surfaces to dissolution, MAPs could have potential as erosion-preventive agents.

The purpose of the present paper was therefore to evaluate whether MAPs inhibit dissolution of HA surfaces. The substrate chosen was compressed HA in disc form. These have been used in previous studies [e.g. Barbour et al., 2005] as a model for dental tissues in studies of other inhibitors and have been shown to react to solution factors such as pH in a qualitatively similar way as enamel [Shellis et al., 2010]. For comparison, the effect of fluoride treatment was also studied. In vivo, salivary proteins readily adsorb to tooth surfaces and form a pellicle: a film which has been shown to reduce dissolution in acid [Zahradnik et al., 1976; Hannig and Balz, 1999, 2001; Maupomé et al., 1999]. Therefore, the effects of MAPs on dissolution of both native surfaces and of surfaces which had been previously coated with saliva were studied.

Materials and Methods

Materials

Discs of compressed HA, mean diameter 12.1 mm, mean thickness 1.23 mm, were purchased from HiMed Inc., Old Bethpage, N.Y., USA. Potassium dodecyl phosphate (PDP) was purchased from Rhodia (Paris, France) and sodium dodecyl phosphate (SDP) from Nikko Chemicals Co. (Tokyo, Japan). Sodium fluoride and other reagents were obtained from Sigma-Aldrich (Poole, Dorset, UK). Solutions were prepared using de-ionised water.

Measurement of Dissolution Rate

Dissolution in 14 mM citric acid, pH 3.2, was measured using a pH-stat (718 Stat Titrino: Metrohm UK, Runcorn, Cheshire, UK) with a 50-ml double-walled glass reaction vessel fitted with a lid with 3 inlet ports. Water was pumped by a circulating water bath (Type GD120; Grant Instruments, Cambridge, UK) through the water jacket to maintain the reaction temperature at 36°C. Prior to use in the pH-stat, batches of discs were conditioned to ensure consistency of response. The discs were first ultrasonicated in de-ionised water in a bath with an ultrasonic power of 40-kHz for 15 min to remove loose HA particles. They were then exposed to gently stirred 14 mM citric acid, pH 3.2, for 30 min at room temperature, then washed in de-ionised water and finally air-dried. For use in the pH-stat, the back of the discs were coated with nail varnish to leave an area of 161.4 mm^2 available for reaction. The disc was then fixed with sticky wax to the tip of a glass tube fitted with a cone suitable for the inlet ports.

In each experiment, 30 ml of citric acid solution was introduced into the reaction vessel and the pH electrode and burette tip fitted. After the system had reached thermal equilibrium, the pH was adjusted to 3.2 by adding small quantities of concentrated KOH or HCl solution and finally adjusted using the pH-stat. The reaction was initiated by introducing the specimen on its holder and addition of titrant (50 mM HCl) was recorded for 30 min. A control measurement of dissolution rate was made after the conditioning step and the disc was removed from the holder, washed and dried. After exposing the disc to the chosen treatment, the disc was reattached to the glass specimen holder and a second measurement of dissolution rate was made.

Saliva Collection

Mixed saliva was collected from a panel of 6 volunteers. When saliva was required, each volunteer was provided with a 50-ml polystyrene Universal tube, marked at the 25-ml level. Each volunteer chewed a square of Parafilm to stimulate salivary flow and expectorated saliva into the tube until the mark was reached. These samples were combined and centrifuged using a Centaur 1 (MSE, London, UK) at 4,000 *g* for 15 min at ambient temperature. The supernatant was used to treat HA specimens.

Experiments

Native HA. In the first series of experiments, inhibition was tested on non-saliva-treated HA, using a variant of the method of Barbour et al. [2005]. First, a sequence of three control measurements was performed. Next, the HA disc was immersed in MAP or fluoride solution for 2 min with gentle stirring. Sodium fluoride was used at 7.89 mM (equivalent to 300 mg/l fluoride). PDP was used at concentrations of 0.01% w/v, 0.1% w/v and 1% w/v. SDP,

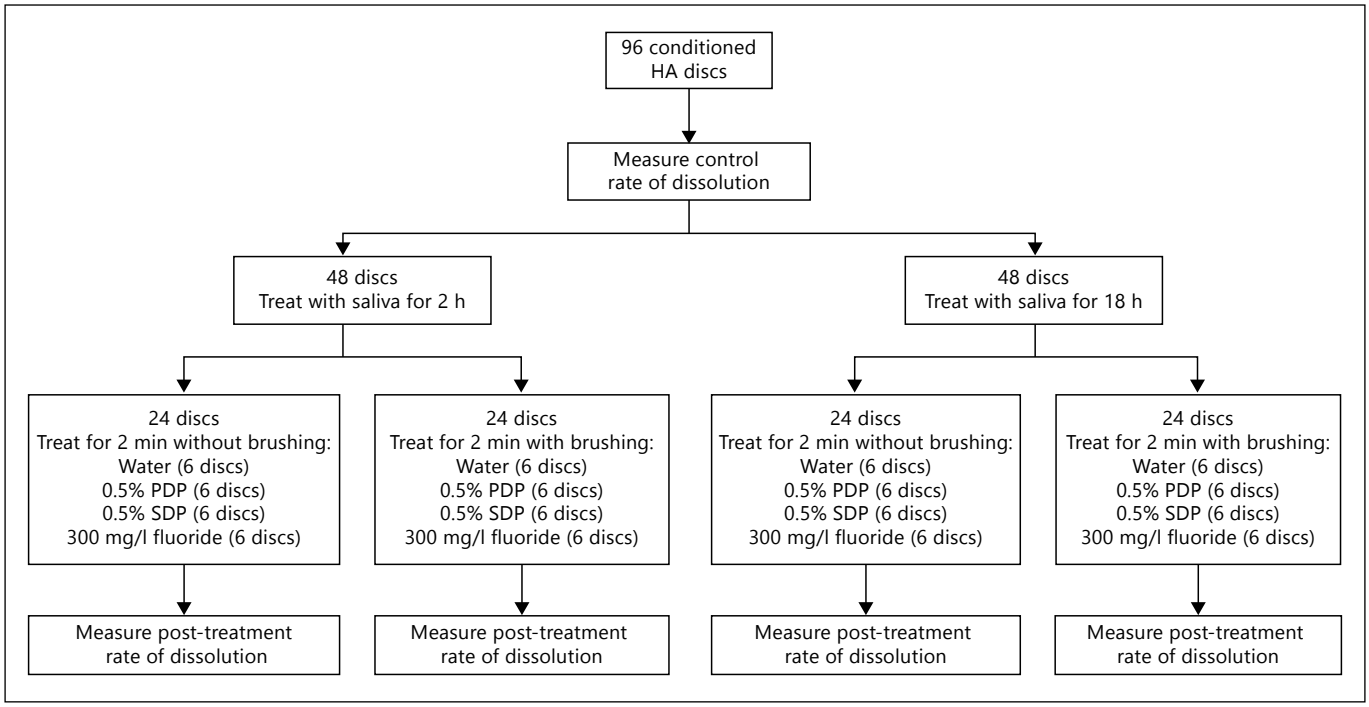

Fig. 1. Flow chart showing the design of the experiment on saliva-coated HA.

being insoluble at 1% w/v, was used only at 0.01% w/v and 0.1% w/v. After rinsing in water, a series of up to 7 post-treatment measurements of dissolution was performed. The number of post-treatment measurements varied between experiments, as they were terminated once the dissolution rate had returned to pre-treatment control levels. This sequence was performed on 6–8 separate HA discs for each treatment.

Saliva-Coated HA. Experiments to test the agents on saliva-treated HA used the same concentration of fluoride (300 mg/l) as in those on native HA, but the concentration of the MAPs was 0.5% w/v. This was the highest concentration available for SDP and it was necessary to heat the 0.5% SDP solution to 50°C to facilitate dissolution, before then allowing it to cool to ambient temperature.

These experiments are summarised in figure 1. Between each step, the discs were rinsed and stored in water. After making control measurements of dissolution rate, discs were immersed in pooled mixed saliva supernatant for 2 or 18 h in Petri dishes which were agitated by an orbital shaker in an incubator at 36°C. The discs were then treated, in groups of 6, with either water (control), MAP or fluoride for 2 min, by one of two methods. Treatments were applied to non-brushed discs for 120 s under gentle agitation. In the remaining brushed discs, the treatments were applied while being brushed with an Aquafresh Powerclean toothbrush (GSK, Weybridge, Surrey, UK) for 10 s using a force of 0.50 N. Sticky wax was used to hold the specimen in place on the base of a weighing boat, 33 ml of solution was added to the weighing boat and the force of the brushing was standardised by placing the weighing boat on the platform of a balance. Following brushing, the disc remained immersed in the solution for a further 110 s with gentle agitation. Finally, the dissolution rate was re-measured on each disc.

Chemical Calculation

Calculations to assess whether calcium fluoride formation at the surface of the HA discs during treatment with NaF solution was possible were performed using an ion speciation program [Shellis, 1988]. It was assumed that, during treatment with NaF, the solution immediately adjacent to the HA surface had a pH of 7.0 and was saturated with respect to HA in the presence of 300 mg/l F and that the solid consisted of stoichiometric HA, i.e. with a Ca/P ratio of 5/3, and had the solubility reported by McDowell et al. [1977].

Statistical Analysis

Preliminary Tests. All data were tested for normality using the Shapiro-Wilks test (XLSTAT software, version 7.5; Addinsoft, Brooklyn, N.Y., USA). Only one dataset showed significant non-normality and this was rectified by omission of one outlier. Prior to analysis of covariance, the extent of correlation between the reduction in dissolution rate and the respective control rate was assessed using Pearson's correlation coefficient (r). In all statistical tests, the experimentwise probability for significance was set at 0.05.

Native HA. A control value for each specimen was calculated by averaging the three pre-treatment runs. Two analyses were performed: the first to study the persistence of action of each treatment, the second to compare the size of the reduction between treatments. In the first analysis, the control means and each suc-

cessive post-treatment mean were compared within each experiment by repeated-measures analysis of variance with a Greenhouse-Geisser correction where necessary, using PASW statistics software (version 18). When this analysis indicated a significant variation between runs, the post-treatment values were compared with the mean control values using paired t tests (MS Excel 2007). The significance level was adjusted using the Dunn-Sidak correction, based on the number of post-treatment runs. In the second analysis, for each experiment the mean maximum reduction in dissolution (control rate – first post-treatment rate) was first calculated. The reduction in rate was correlated with variations in the control rate ($r = 0.86$; $p < 0.001$). Therefore, the mean reductions were subjected to analysis of covariance [Vickers, 2001], with control rate as a quantitative factor and the treatment (fluoride/0.1% SDP/0.1% PDP/1% PDP) as a qualitative factor. Post hoc pairwise comparisons were made using Tukey and REGWQ tests (XLSTAT).

Saliva-Coated HA. Initial tests compared the control and post-treatment rates using paired t tests. In further analysis, the outcome variable was the reduction in dissolution rate (pre-treatment control rate – post-treatment rate). As the reduction was correlated with the control rate ($r = 0.81$; $p < 0.001$), analysis of covariance was employed. To determine whether the inhibition due to saliva treatment was altered by the exposure time or by brushing, the data for specimens that had been treated with water after saliva exposure were subjected to analysis of covariance, with the control rate as a quantitative factor, and the exposure time (2 h/18 h) and brushing (non-brushed/brushed) as qualitative factors. To assess the effect of treatment with the test agents on the inhibition due to saliva, the data for each combination of saliva exposure time and brushing treatment were separately subjected to analysis of covariance with the control rate as a quantitative factor and the treatment (water/SDP/PDP/fluoride) as qualitative factors. Since the question of interest in this analysis was whether treatment with MAP or fluoride affected the dissolution rate of saliva-treated HA, the MAP and fluoride groups were compared with the water (control) group by Dunnett's test. These analyses were performed using XLSTAT.

Results

Native HA

Exposure to 300 mg/l fluoride resulted in a reduction of 12% in dissolution rate ($p = 0.003$), but the reduction did not persist beyond the first post-treatment run (fig. 2; table 1). The equilibrium Ca^{2+} concentration next to the HA surface was estimated to be 0.105 mM, and this region of the solution would therefore be supersaturated with respect to both calcium fluoride (degree of saturation, S = 6.74) and fluorapatite (S = 4.92).

Exposure to either PDP or SDP at 0.01% w/v did not reduce dissolution rate (fig. 3, 4), but both agents reduced dissolution rate ($p < 0.001$) at higher concentrations (fig. 3–5), the maximum inhibition (26–32%) occurring in the first post-treatment run. Comparison of the maximum inhibitions indicated that the reduction due to fluoride was significantly smaller than those due to the MAPs, but there was no clear pattern of significant differences among the MAP treatments (table 1). The dissolution rate remained numerically below the control rate for 4–7 post-treatment runs (fig. 3–5; table 1), indicating persistence of action, but the difference from the control rate remained significant only for 2 runs (0.1% SDP or PDP) or 6 runs (1% PDP) (table 1).

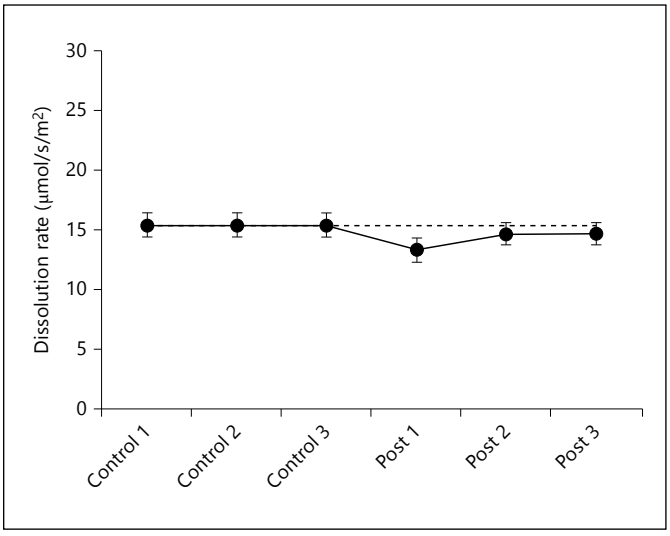

Fig. 2. Dissolution rate of HA discs before (control 1–3) and after (post 1–3) treatment with 300 mg/l fluoride as NaF. Dashed line = Mean control rate.

Table 1. Inhibition of dissolution during sequential experiments with HA not treated with saliva

Agent	Maximum inhibition μmol/m²/s	Number of post-treatment runs showing significant inhibition
Sodium fluoride 300 mg/l	1.97 (1.41)[a]	1
SDP 0.1%	4.97 (1.33)[b]	2
PDP 0.1% w/v	5.90 (0.66)[b, c]	2
PDP 1% w/v	6.26 (1.44)[c]	6

The maximum inhibition (mean, SD in parentheses) is that recorded at the first post-treatment run. Means sharing the same superscript letters are not significantly different. The number of post-treatment runs (including the first) when dissolution was significantly less than pre-treatment control is a measure of the persistence of the inhibitory effect.

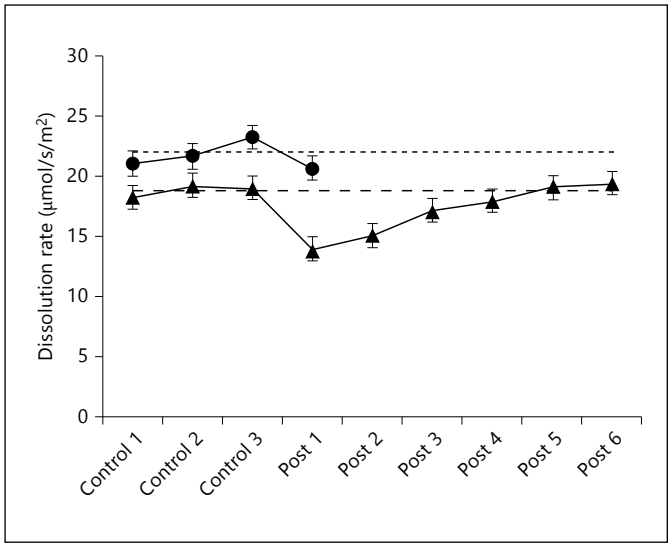

Fig. 3. Dissolution rate of HA discs before (control 1–3) and after (post 1–6) treatment with SDP. Circles = 0.01% SDP; triangles = 0.1% SDP; dotted line = 0.01% mean control rate; dashed line = 0.1% mean control rate.

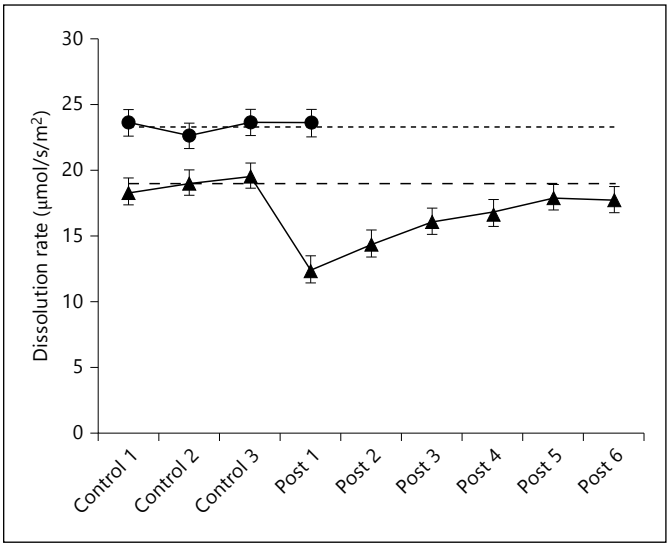

Fig. 4. Dissolution rate of HA discs before (control 1–3) and after (post 1–6) treatment with PDP. Circles = 0.01% SDP; triangles = 0.1% SDP; dotted line = 0.01% mean control rate; dashed line = mean control rate.

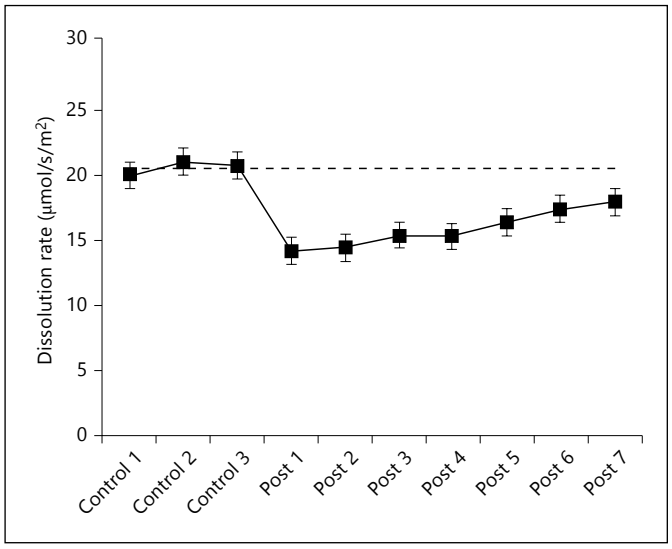

Fig. 5. Dissolution rate of HA discs before (control 1–3) and after (post 1–7) treatment with 1% PDP. Dashed line = 0.1% mean control rate.

Saliva-Treated HA

Figures 6–9 summarise the results of these experiments in terms of the percentage reduction in dissolution rate. In all groups, the dissolution rate after treatment with saliva followed by water or any of the experimental agents was significantly less than the pre-saliva-exposure control rate ($p < 0.001$), except for the 2-hour/non-brushed group treated with PDP ($p = 0.17$).

For water-treated specimens, the reduction in dissolution rate was significantly greater after exposure to saliva for 18 h than after 2-hour exposure ($p < 0.001$). The relative reductions were, on average, 41% (2 h) and 63% (18 h). Brushing did not have a significant effect on dissolution rate in the water-treated specimens ($p = 0.13$).

The treatment was not a significant factor in the 18 h/non-brushed group ($p = 0.13$). Fluoride significantly increased the reduction in dissolution in brushed specimens, after both 2-hour and 18-hour exposure to saliva. In non-brushed 2-hour specimens, and brushed 18-hour specimens, the reduction in dissolution was significantly less after treatment with 0.5% PDP.

Discussion

As HA is the type mineral for dental tissues, the discs used as a substrate in these experiments should have reasonably similar surface properties to dental mineral. In the pH-stat system, HA discs resemble enamel in that dissolution is linear with time and is strongly pH-dependent [Shellis et al., 2010]. Because of its large proportion of collagenous matrix, the pattern of dissolution of dentine is

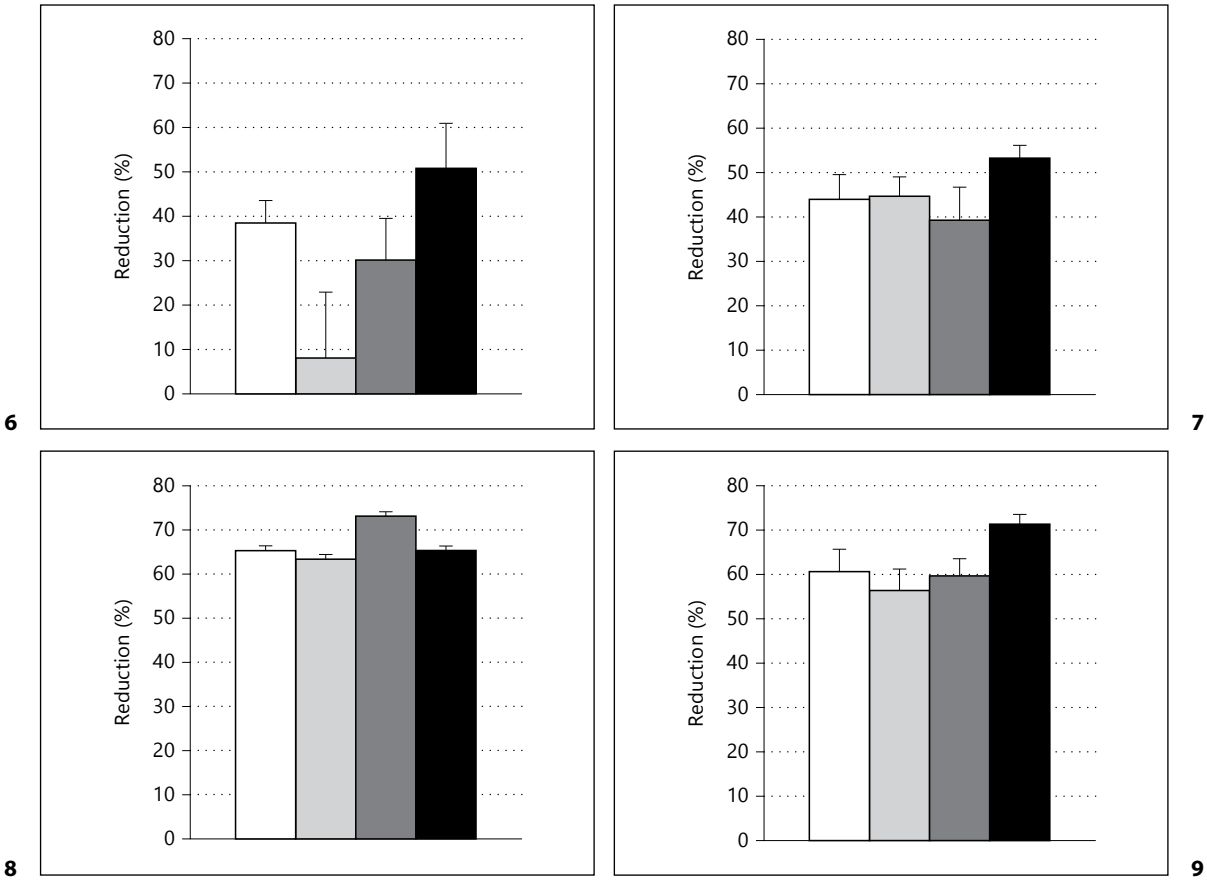

Fig. 6–9. Percent reduction in dissolution rate of HA discs pre-treated with saliva, after subsequent treatment with water (control: white), 0.5% PDP (pale grey), 0.5% SDP (mid grey) or 300 mg/l fluoride as NaF (black). **6** Two-hour saliva treatment, agents applied without brushing. **7** Two-hour saliva treatment, agents applied with brushing. **8** Eighteen-hour saliva treatment, agents applied without brushing. **9** Eighteen-hour saliva treatment, agents applied with brushing.

very different from that of HA and enamel [Shellis et al., 2010; Lussi et al., 2011]. HA discs are therefore a better model for enamel than for dentine. However, they consist of much coarser particles than either enamel or dentine, and contain larger pores [Shellis et al., 2010].

The present results show that the pH-stat technique originally devised to study the effect on erosion of additives to erosive drinks can also be applied successfully to studying the effect of pre-treatments on subsequent erosion. A particularly useful aspect of the method is that it provides information on the persistence of the inhibitory effect during repeated erosive challenges. With few exceptions [e.g. Wiegand et al., 2005], previous studies of topical anti-erosive agents have given information only on the immediate effect, since tests have been confined to one post-treatment challenge.

All of the test substances inhibited dissolution of native HA, but the extent and persistence of the effect varied. The reduction in dissolution rate observed after fluoride treatment was relatively modest. Greater inhibition by NaF (about 25–40%) has been observed in previous in vitro studies on enamel [Davis and Winter, 1977; Ganss et al., 2001, 2011; van Rijkom et al., 2003; Hughes et al., 2004] using longer exposure times and toothpastes or rinses with much higher fluoride concentrations than were used here. The inhibition due to fluoride did not persist beyond the first post-treatment run, indicating that the adsorbed fluoride was removed, or reduced to an ineffective level, by the acid challenge. However, the data of Wiegand et al. [2005] indicate that pre-treatment with higher fluoride concentrations than that used here would have a more long-lasting effect on erosion as well as a

greater immediate effect. It is widely believed that fluoride is protective against acid attack because it induces formation of a layer of KOH-soluble calcium fluoride [Magalhães et al., 2011], although calcium fluoride is solubilised by subsequent acid challenges [Ganss et al., 2007]. Although our calculation indicates that calcium fluoride formation at the HA surfaces is theoretically possible, the degree of saturation was relatively modest and it seems very likely that the amount of calcium fluoride formed during the 2-min low-fluoride treatment would be small and easily removed after one post-test run. Although the solution at the HA surface was also supersaturated with respect to fluorapatite, precipitation of this solid seems less likely, given the low degree of supersaturation. Moreover, Cruz et al. [1992] could not detect fluorapatite formation even after exposure to much higher concentrations of fluoride (9,048 mg/l).

After the brief exposure used in our experiments, the MAPs showed much greater inhibitory activity than a concentration of fluoride typical of many mouth rinses, and, moreover, showed some persistence of action. These differences from fluoride may be due solely to the low fluoride concentration used, as published data (see above) suggest that fluoride at higher concentrations could have comparable efficacy to the MAPs and also show persistence of action.

MAP anions adsorb strongly to HA surfaces by co-ordination between the phosphate head group and surface Ca^{2+} ions [Tanaka et al., 1997a, b]. We assume that the inhibitory effect of MAPs on dissolution is due to adsorption at high-energy dissolution sites on the crystal surfaces. MAP adsorption would also make the crystal surfaces as a whole more hydrophobic. It was striking that 1% PDP did not significantly increase the immediate inhibitory effect over 0.1% PDP but increased the persistence of action considerably (table 1). It is possible that 0.1% PDP is sufficient to saturate the dissolution sites and that at higher concentrations the excess PDP is adsorbed to the general crystal surface and provides a reservoir which can replace PDP released from dissolution sites during repeated post-treatment exposures to acid solution.

Our second set of experiments agrees with numerous previous reports which show that exposure of dental tissues to saliva inhibits dissolution by acidic solutions [Zahradnik et al., 1976; Hannig and Balz, 1999, 2001; Maupomé et al., 1999]. Some studies indicate that exposure time has no significant effect [Maupomé et al., 1999; Hannig et al., 2003, 2004], but Wetton et al. [2006] found that a statistically significant effect in vitro was obtained only for exposure times of ≥60 min. For this reason we used a minimum exposure time of 2 h to ensure an effect on HA discs, but found that there was a significant further increase in inhibitory effect between 2 and 18 h. This could be due to an increase in thickness or to alteration of the properties of the adsorbed salivary film over time. There is, however, an alternative explanation. The outer region (about 30 μm deep) of acid-conditioned discs has enhanced porosity, since it consists of partly dissolved HA particles separated by wide pores [Shellis et al., 2010, fig. 3B]. In control runs, the recorded dissolution is due to loss of mineral throughout this layer. During saliva treatment, it can be assumed that salivary proteins diffuse to a certain depth, adsorb to the HA surfaces and inhibit dissolution. Longer exposure would therefore have a greater effect because saliva could diffuse further into the porous outer layer of the discs and coat particles deeper in the structure, hence inhibiting dissolution from a larger proportion of the accessible HA particles.

The hypothesis that saliva treatment affects dissolution of HA particles for some depth in the HA discs has implications for interpreting the results of experiments involving further treatments after saliva treatment. The short duration of the post-saliva treatments would probably limit the depth to which agents could diffuse into the porous surface zone, so the reactivity of the deeper parts of the zone would be unaltered. A similar explanation can be offered for the lack of effect of brushing. It is known that brushing removes most, although not all, of the salivary pellicle formed on enamel in situ, even when toothpaste is not used [Joiner et al., 2008]. If, however, this effect is limited to the surface of the disc, the overall effect on dissolution rate would be small or undetectable as particles below the surface would remain protected by saliva. While experimental models using HA discs as substrates are valuable for studying the effects of single treatments, depth-related phenomena such as these would reduce their sensitivity for studying the effect of saliva in combination with other treatments, especially those which depend on surface adsorption.

Pre-treatment with saliva modified the effects of the test agents considerably. Fluoride had the smallest effect on dissolution rate of native HA discs, had no effect on dissolution of saliva-coated discs when applied without brushing but increased the effect of saliva when applied with brushing. In the latter cases brushing would have removed at least part of the superficial salivary film and hence facilitated inward diffusion of fluoride and formation of KOH-soluble calcium fluoride at the HA surfaces. As KOH-soluble calcium fluoride precipitation is not a surface adsorption process it would not interfere with the

residual salivary coating. In contrast, the undisturbed salivary film on non-brushed surfaces appears to hinder reaction of fluoride with the HA surface.

While the MAPs showed strong, consistent inhibition of dissolution of native HA surfaces, they had in most cases no significant effect on the dissolution rate of saliva-coated HA. In other words, there was no additive effect of saliva and MAPs. This does not, however, imply a lack of interaction between the MAPs and the HA surface. MAP could adsorb to the HA surface without displacing salivary protein: this is likely to enhance the reduction in dissolution rate. On the other hand, adsorption of MAPs to the HA surface with simultaneous displacement of salivary protein would probably result in an increase in dissolution rate, as the effect of MAPs on native HA dissolution is less than that of salivary films. As both types of adsorption are possible on the same disc, and as the effect of MAPs on HA dissolution is relatively large (approximately 50–75% of that of salivary films), the net effect of the interaction between MAPs and saliva-coated discs could well be too small to be detected.

The abolition of the inhibitory effect of saliva on HA dissolution by PDP on 2-hour/non-brushed discs suggests that it is possible for this MAP to desorb salivary protein without itself adsorbing to the HA surface. However, it is not possible to explain why a similar effect was observed on discs with disparate saliva exposure time and brushing treatment, nor the lack of this effect with SDP, which differs from PDP only in the counter-ion. The interaction of MAPs with saliva-coated HA surfaces would be worth further investigation to understand the various effects on HA dissolution.

The experiments on saliva-treated HA clearly did not support the conclusion, from the experiments on native HA, that MAPs are potentially highly effective anti-erosion agents. However, further work would be worthwhile. MAPs could be effective against erosion of saliva-coated surfaces which have been subjected to more prolonged brushing, or which have been brushed with a dentifrice. Greater inhibition might also be observed with enamel, in which erosion is not complicated by the subsurface effects which appear to influence the behaviour of HA discs.

Although the results of the present study are inconclusive with respect to the efficacies of MAPs in erosion prevention, it is an important finding that saliva has such a strong influence on tests of anti-erosive agents. We suggest that screening of such agents should always include specimens that have been pre-treated with saliva as well as native surfaces.

Acknowledgements

We thank Glaxo SmithKline plc for financial support to Dr. Jones.

References

Barbour ME, Shellis RP, Parker DM, Allen GC, Addy M: An investigation of some food approved polymers as agents to inhibit hydroxyapatite dissolution. Eur J Oral Sci 2005;113:457–461.

Cruz R, Ogaard B, Rölla G: Uptake of KOH-soluble and KOH-insoluble fluoride in sound human enamel after topical application of a fluoride varnish (Duraphat) or a neutral 2% NaF solution in vitro. Scand J Dent Res 1992;100:154–158.

Davis WB, Winter PJ: Dietary erosion of adult dentine and enamel: protection with a fluoride toothpaste. Br Dent J 1977;143:116–119.

Ganss C, Klimek J, Brune V, Schürmann A: Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res 2004;38:561–566.

Ganss C, Klimek J, Schäffer U, Spall T: Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. Caries Res 2001;35:325–330.

Ganss C, Lussi A, Grunau O, Klimek J, Schlueter N: Conventional and anti-erosion fluoride toothpastes: effect on enamel erosion and erosion-abrasion. Caries Res 2011;45:581–589.

Ganss C, Neutard L, von Hinckeldey J, Klimek J, Schlueter N: Efficacy of a tin/fluoride rinse: a randomized in situ trial on erosion. J Dent Res 2010;89:1214–1218.

Ganss C, Schlueter N, Klimek J: Retention of KOH-soluble fluoride on enamel and dentine under erosive conditions – a comparison of in vitro and in situ results. Arch Oral Biol 2007;52:9–14.

Hannig M, Balz M: Influence of in vivo formed salivary pellicle on enamel erosion. Caries Res 1999;33:372–379.

Hannig M, Balz M: Protective properties of salivary pellicles from two different intraoral sites on enamel erosion. Caries Res 2001;35:142–148.

Hannig M, Fiebiger M, Güntzer M, Döbert A, Zimehl R, Nekrashevych Y: Protective effect of the in situ formed short-term salivary pellicle. Arch Oral Biol 2004;49:903–910.

Hannig M, Hess NJ, Hoth-Hannig W, De Vrese M: Influence of salivary pellicle formation time on enamel demineralization – an in situ pilot study. Clin Oral Investig 2003;7:158–161.

Hughes JA, West NX, Addy M: The protective effect of fluoride treatments against enamel erosion in vitro. J Oral Rehabil 2004;31:357–363.

Ishikawa T, Tanaka H, Yasukawa A, Kandori K: Modification of calcium hydroxyapatite using ethyl phosphates. J Mater Chem 1995;5:1963–1967.

Jaeggi T, Lussi A: Prevalence, incidence and distribution of erosion; in Lussi A (ed): Dental Erosion. Monogr Oral Sci. Basel, Karger, 2006, vol 20, pp 44–65.

Joiner A, Schwarz A, Philpotts CJ, Cox TF, Huber K, Hannig M: The protective nature of pellicle towards toothpaste abrasion on enamel and dentine. J Dent 2008;36:360–368.

Lussi A, Schlueter N, Rakhmatullina E, Ganss C: Dental erosion – an overview with emphasis on chemical and histopathological aspects. Caries Res 2011;45(suppl 1):33–42.

Magalhães AC, Wiegand A, Rios D, Buzalaf MA, Lussi A: Fluoride in dental erosion; in Buzalaf MA (ed): Fluoride in the Oral Cavity. Monogr Oral Sci. Basel, Karger, 2011, vol 22, pp 158–170.

Maupomé G, Aguilar-Avila M, Medrano-Ugalde H, Borges-Yáñez A: In vitro quantitative microhardness assessment of enamel with early salivary pellicles after exposure to an eroding cola drink. Caries Res 1999;33:140–147.

McDowell H, Gregory TM, Brown WE: Solubility of $Ca_5(PO_4)_3OH$ in the system $Ca(OH)_2$-H_3PO_4-H_2O at 5, 15, 25 and 37°C. J Res Natl Bur Stds 1977;A81:273–281.

McGaughey C, Stowell EC: Effects of polyphosphates on the solubility and mineralization of HA: relevance to rationale for anticaries activity. J Dent Res 1977;56:579–587.

Schaad P, Thomann JM, Voegel JC, Gramain P: Inhibition of dissolution of hydroxyapatite powder by adsorbed anionic polymers. Colloids Surf A: Physiochem Eng Aspects 1994; 83:285–292.

Shellis RP: A microcomputer program to evaluate the saturation of complex solutions with respect to biominerals. Computer Appl Biosci 1988;4:373–379.

Shellis RP, Barbour ME, Jones SB, Addy M: Effects of pH and acid concentration on erosive dissolution of enamel, dentine and compressed hydroxyapatite. Eur J Oral Sci 2010; 118:475–482.

Tanaka H, Yasukawa A, Kandori K, Ishikawa T: Surface modification of calcium hydroxyapatite with hexyl and decyl phosphates. Colloids Surf A. Physiochem Eng Aspects 1997a;125:53–62.

Tanaka H, Yasukawa A, Kandori K, Ishikawa T: Modification of calcium hydroxyapatite using alkyl phosphates. Langmuir 1997b;13:821–826.

van Rijkom H, Ruben J, Vieira A, Huysmans M-CDNJM, Truin G-J, Mulder J: Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride in vitro. Eur J Oral Sci 2003;111: 253–257.

Vickers AJ: The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. BMC Med Res Methodol 2001;1:6–9.

Wetton S, Hughes J, West N, Addy M: Exposure time of enamel and dentine to saliva for protection against erosion: a study in vitro. Caries Res 2006;40:213–217.

Wiegand A, Attin T: Influence of fluoride on the prevention of erosive lesions – a review. Oral Health Prev Dent 2003;1:245–253.

Wiegand A, Krieger C, Attin R, Hellwig E, Attin T: Fluoride uptake and resistance to further demineralisation of demineralised enamel after application of differently concentrated acidulated sodium fluoride gels. Clin Oral Investig 2005;9:52–57.

Wiegand A, Magalhães AC, Attin T: Is titanium tetrafluoride (TiF_4) effective to prevent carious and erosive lesions? A review of the literature. Oral Health Prev Dent 2010;8:159–164.

Zahradnik RT, Moreno EC, Burke EJ: Effect of salivary pellicle on enamel subsurface demineralization in vitro. J Dent Res 1976;55:664–670.