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CPP–ACP and CPP–ACFP *versus* fluoride varnish in remineralisation of early caries lesions. A prospective study

ABSTRACT

Aim *To evaluate the effects of novel casein phosphopeptide (CPP) formulations CPP-amorphous calcium phosphate (CCP-ACP) and CPP-amorphous calcium fluoride phosphate (CPP-ACFP) versus fluoride varnish on the remineralisation of enamel white spot lesions (WSLs) over a 12-week follow-up period.*

Materials and Methods *Study Design: Double-blinded prospective study. Eligibility criteria were patients between 6 and 14 years old and have WSLs on their permanent teeth. We evaluated 786 WSLs. Participants were divided randomly into three groups. Groups A and B were instructed to daily topical application of GC Tooth Mouse (CPP-ACP) or Mi Paste Plus (CPP-ACFP), Group C received a monthly professional application of Duraphat fluoride varnish. WSLs were categorised according to the International Caries Detection and Assessment System (ICDAS II; grades 0–3) and assessed by laser fluorescence (DIAGNOdent) at baseline and at 4, 8 and 12 weeks. Changes in mineralisation before and after treatment were analysed using two-way analysis of variance, with post hoc Bonferroni's non-parametric tests for multiple comparisons.*

Results *DIAGNOdent values were significantly reduced in Group B at 4 weeks, and in Groups A and C at 8 weeks. Mean values in Group B were lower than in Groups A and C at 4 weeks, and lower than Group C at 8 weeks. CPP-ACFP appeared to have a specific effect on smooth-surface caries, but no significant effect on caries in pits and fissures.*

Conclusions *At 4 weeks, CPP-ACFP is superior to fluoride varnish at remineralising smooth-surface WSLs. CPP-ACP is not superior to fluoride varnish by any of the measures studied.*

Keywords *Casein phosphopeptide amorphous calcium phosphate; Casein phosphopeptide amorphous calcium fluoride phosphates; Dental caries; Fluoride varnish; Laser fluorescence; White spot lesion.*

Introduction

Dental caries is the progressive demineralisation of tooth enamel that occurs when plaque bacteria metabolise dietary sugars into acids that liberate calcium and phosphate ions from hydroxyapatite (Hap). Caries is a highly prevalent disease across the world and, although its prevalence has declined, the disease remains a major public health problem [Selwitz, 2007]. Pathologically, caries lesions develop gradually, starting as a minimal structural change that is not detectable clinically, progressing to a clinically visible patch of demineralisation, and onwards to inflict increasing degrees of tissue destruction upon the tooth. However, at the early stages of the disease process, the relationship between pathogenic and protective factors is dynamic and thoroughly reversible [Rao et al., 2009]. For maximally effective remineralisation, calcium, phosphate and fluoride must all be available at sufficient levels in a bioavailable form in the oral environment [Reynolds et al., 2008; ten Cate et al., 2008].

Application of high-concentration fluoride preparations creates a superficial reserve of calcium fluoride, from which fluoride is released when the oral pH drops, thus maximising remineralisation in the aftermath of a carious attack. However, an ideal remineralisation system should include not only fluoride but also bioavailable calcium and phosphate to produce a more comprehensive sub-superficial remineralisation [Willmot, 2004].

Various preparations containing compounds based on bioavailable phosphate and calcium have been investigated, usually containing between 1–3 mM calcium ions, with phosphate ions present at a ratio of 1:1 [Wefel and Harless, 1987] or 1.66:1 [Iijima et al., 1999], some of them also contain fluoride. These can be carried by a vehicle prepared from bovine milk, which is a good source of phosphopeptide-based products for dentistry use in humans. These phosphopeptide-based preparations are of three main types: casein phosphopeptides (CPP); casein phosphopeptides with amorphous calcium phosphate (CPP-ACP); and casein phosphopeptides with amorphous calcium fluoride phosphate (CPP-ACFP) [Aimutis, 2004].The efficacy of

CPP-ACP and CPP-ACFP in prevent demineralisation and promote remineralisation of early enamel lesions has been demonstrated in several in vitro, in situ and in vivo studies [Cochrane, et al., 2008; Shen et al., 2001; Cai et al., 2007; Morgan et al., 2008; Ferrazzano et al., 2007; Kargul et al., 2012]. Various randomised-controlled clinical trials of post-orthodontic WSL regression following the use of CPP-ACP preparations have been reported [Andersson et al., 2007; Bailey et al., 2009; Bröchner et al,2011]. However, other studies could not demonstrate a conclusive benefit of CPP-ACP-based remineralisation products [Beerens et al., 2010; Huang et al., 2013].

In an effort to resolve the uncertainty surrounding the usefulness of these types of products, the aim of this study was therefore to perform a double-blinded prospective study to compare the relative remineralising effect of CCP-ACP and CPP-ACFP formulations versus a 5% fluoride varnish on the remineralisation of enamel WSLs over a 12-week follow-up period. The null hypothesis was that the remineralisation efficacy of CPP-ACP and CPP-ACFP over a period of three months would be no different to that of a 22,600 ppm fluoride varnish. The study has been designed to conform as far as possible to CONSORT guidelines on clinical trial design [Moher et al., 2003].

Materials and Methods

This double-blinded prospective study took place in Valencia, Spain over the period between February and September, 2012. Eligibility criteria were that the participants must: 1) be male or female children between the ages of 6 and 14 years; 2) be attending a Primary Dental Care facility for assessment for the first time; 3) have one or more WSLs in their permanent teeth, visible with or without prolonged air drying as a distinct visual change in the enamel and or localised enamel breakdown but without visible clinical signs of dentinal involvement; 4) live in an area where the water supply was non-fluoridated; and 5) be otherwise medically fit and well, with no systemic diseases, syndromic abnormalities, or proven/suspected milk protein allergy and/or sensitivity, or allergy to benzoate preservatives. Patients failing to satisfy all of these inclusion criteria were rejected. Informed written consent for the participation of all eligible participants was obtained from their parent/guardians following explanation of the intended benefits and potential risks of the study protocol. The study design was approved by the medical ethics committee at the University General Hospital Foundation of Valencia, Spain (approval number: 029/2008).

The study was initiated to compare the relative remineralising effects of self-administered CPP-ACP paste (GC Tooth Mousse®, Recaldent-GC, Leuven, Belgium), group A, self-administered CPP-ACFP paste containing CPP-ACP plus 900 ppm (0.2% w/w) sodium fluoride (MI Paste Plus®, Recaldent-GC, Leuven, Belgium), group B, and a professionally-applied 5% sodium fluoride varnish (Duraphat®, Colgate Palmolive GmbH, Hamburg, Germany), Group C.

Verbal and written usual oral hygiene instructions were given to the participants and to the responsible adult to facilitate and reinforce compliance. Also each participant received a packet with nonprescription fluoride toothpaste (1100 ppm of fluoride), and a manual toothbrush. Instructions were also given that other sources of fluoride (environmental, supplements, professional, or other dental products) should be avoided during the study period. Compliance was checked at each progress visit to assess the frequency of brushing and application of the study paste in groups A and B. Furthermore, subjects were asked to bring their study paste to each visit to monitor usage level.

Self-administration in Groups A and B was standardized by giving specific instructions to each participant, stipulating that the product should be used once daily (in the evening), after a 2 min manual tooth brushing. A pea-size amount of the product should be applied per arch, using a dry finger or cotton pellet to distribute it evenly across all teeth and to work it into the interdental spaces. The product was then retained in the mouth for 1–3 min, and manipulated around the teeth using the tongue, before being expectorated. Participants were explicitly instructed not to rinse their mouths after application and not to eat or drink for at least 30 min.

Following recruitment, baseline characteristics were assessed. Teeth were cleaned with a prophylaxis brush without paste. Using a dental light reflector, visual inspection of all tooth surfaces for WSLs was undertaken, initially with wet tooth surfaces and again after 5 s of drying with a gentle air stream. Multiple lesions could be recorded per tooth. Each lesion was scored according to ICDAS II criteria [Topping and Pitts, 2009] for severity, selecting only lesions scored as 1, 2 or 3; activity was assessed by the Ekstrand criteria [Ekstrand et al., 2009]. Examinations were conducted by a single dentist with considerable clinical experience. Following two sessions of training and standardisation with written and visual instructions and live calibration participants, we determined that the intra-examiner kappa value (i.e. the reproducibility of these examinations) was 0.92.

Laser fluorescence (LF) examination for each lesion was performed using DIAGNOdent (Kavo, Biberach, Germany). It operates with a diode laser having a wavelength of 655 nm and 1 mW peak power. Sound enamel does not fluoresce at this wavelength, but caries and bacteria do. Probe A was used for the occlusal surfaces because of its comparatively narrow

tip, which allowed superior access into the pits and fissures, whereas the wider-tipped. Probe B was used for the smooth surfaces. The instrument was calibrated according to manufacturer's instructions. Under cotton roll isolation and after air drying with an air syringe, the DIAGNOdent probe was placed perpendicular to the test site and rotated along the lesion to scan the area completely. Three measurements were taken and averaged to give the final test value. Measurements were taken at baseline and at 4, 8, and 12 weeks after starting treatment. All measurements were conducted by a single dentist; calibration with a standard sample gave an intra-examiner kappa value of 0.89.

We calculated that, to detect a 20% difference between the fluoride varnish and CPP intervention groups for a two-sided test with 5% significance level, the estimated number of WSLs required per group was 246 (assuming that lesions are independent).

In a previous pilot study, the mean number of lesions per child was 13. Therefore, assuming a 5% loss of WSLs throughout the study, we recruited 20 participants to each group. Subjects satisfying the inclusion criteria were then randomly assigned to the three groups. Non-biased randomisation was achieved using a computerised random number list that was generated and locked prior to the start of the study and could not be modified after study initiation (EpiInfo; created by the Centers for Disease Control and Prevention, Atlanta, GA, USA).

Participants in groups A and B were blinded to the group to which they had been assigned and received an unmarked toothpaste tube containing the product corresponding to their allotted group. Group C, in which participants received a monthly professional application of fluoride varnish, could not be blinded owing to the need to attend the clinic for treatment. figure 1 shows a flow chart tracing the treatment process for each group.

The dentist who conducted examinations in each point of the study was blinded for the treatment group.

Data were analysed using SPSS for Windows software, v19 (IBM). Quantitative data presented as the mean and confidence interval. Data were analysed using two-way analysis of variance, with post hoc Bonferroni's non-parametric tests for multiple comparisons. A statistically significant difference was accepted at $p<0.05$. This statistical analysis was approved by an expert statistician.

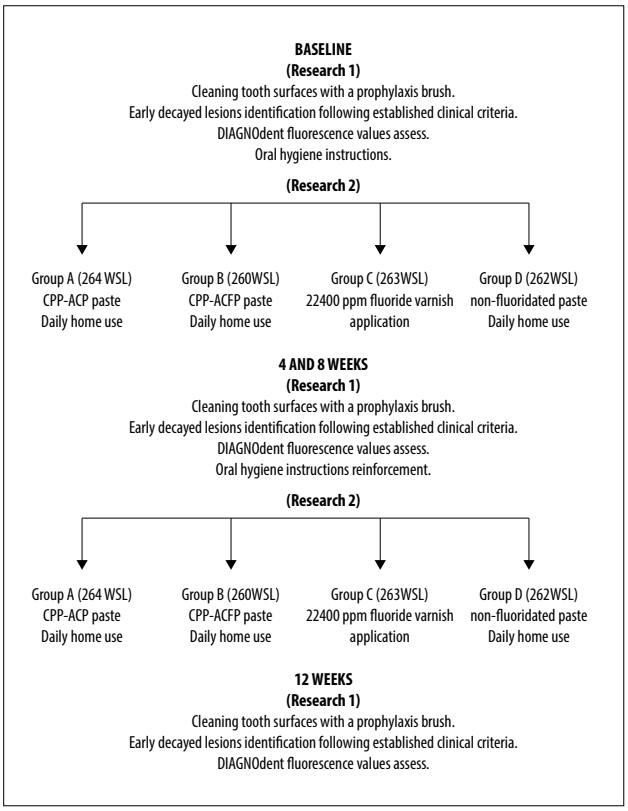

FIG. 1 Treatment process for each study group.

Results

The study sample originally included 786 discrete WSLs subdivided into three groups to receive CPP-ACP (Group A), CPP-ACFP (Group B) or fluoride varnish (Group C), but a small number of drop-outs during the trial meant that the final number analysed across the sample was 775 (Fig. 1). On assessing DIAGNOdent scores at baseline and again at 4, 8 and 12 weeks after treatment initiation, we found that these scores tended downwards in all groups (Table 1). This difference was significant in all groups, but only became so at different time points (12 weeks in Group A, 4 weeks in Group B and 8 weeks in Group C), suggesting that the onset of action may be most rapid in Group B and slowest in

	Group A mean (SD)	Group B mean (SD)	Group C mean (SD)	Group D mean (SD)
Baseline	4.91 (3.28) a,b	4.70(3.42) a.b,c.	5.23 (4.47) a,b	4.44 (3.95)
4 weeks	4.79 (3.48)	3.76 (2.21) a	4.75 (3.37)	4.44 (3.22)
8 weeks	4.03 (3.63) a	3.10 (3.18) b	4.21 (3.20) a	4.05 (2.25)
12 weeks	3.77 (3.33) b	3.12 (3.11) c	4.09 (3.60) b	3.96 (2.31)

Mean (standard deviation). Values sharing the same upper case letter by column are significantly different
Group A: CPP-ACP; Group B: CPP.ACFP; Group C: Fluoride varnish; Group D: control.

TABLE 1 Values of laser fluorescence throughout the study period.

Group A. The decline in DIAGNOdent scores in Group B was particularly clear, with highly significant differences ($p<0.001$) at each time point compared with baseline. Given these differences, it was not surprising that there were inter-group differences at each time point, with scores in Group B being significantly lower at both four and eight weeks than those in Groups A and C at the same time points. Similarly, scores in Group B were significantly lower than in Group C at 12 weeks.

We then investigated whether any of the products had differential efficacy depending on whether they were used on lesions within the pits and fissures of the occlusal surface or on the smooth vertical surfaces of the tooth crown. Results for these analyses are shown in Table 2. Surprisingly, no treatment was particularly effective in the pits and fissures, with small (but nevertheless significant) differences at eight weeks in Groups A and C compared with their respective baseline values. No other significant differences were evident. Conversely, there were both treatment- and time-related decreases in DIAGNOdent values derived from smooth surface lesions. In all groups, values were significantly different ($P<0.01$) to baseline values at eight and twelve weeks. Furthermore, at eight and twelve weeks, scores in Groups A and B were significantly lower than in Group C. However, there was also a small but significant difference in the baseline value in Group C compared with those in Groups A and B.

To compare the relative effects of the three treatments on more severe WSLs, we analyzed the effects of the CPP preparations and fluoride varnish on WSLs with initial DIAGNOdent scores of 10 or more (Table 3). The fluoride varnish group (Group C) exhibited large and significant reductions at all four time points after application, and Group A caused a similarly large but comparatively delayed reduction, which was not apparent until eight weeks. Results in Group B were equivocal, with a somewhat smaller drop in DIAGNOdent scores and fluctuation in and out of statistical significance. This may suggest that the effects of the CPP-ACFP treatment in severe lesions were on the borderline of significance. The only significant difference between the groups at a given time point was that between Groups A and B at four weeks.

	Group A (CPP-ACP)			
	Baseline	4 weeks	8 weeks	12 weeks
	Mean(SD)	Mean (SD)	Media	Media
Pit and fissures	6.29 (4.18)[a,b]	6.14 (3.18)	5.03 (3.50)[a]	5.44 (3.71)[b]
Smooth surface	3.61 (2.89)[a,b]	3.52 (1.52)	2.59 (2.10)[a]	2.70 (2.10)[b]
	Group B (CPP-ACFP)			
Pit and fissures	5.95 (3.71)	5.35 (3.45)	5,71 (3.41)	5.60 (3.45)
Smooth surface	3.72 (2.02)[a,b,c]	3.07 (2.52)[a]	2.53 (1.34)[b]	2.07 (0.75)[c]
	Group C (Fluoride varnish)			
Pit and fissures	6.40 (3.78)[a]	5.75 (3.21)	4.81 (3.54)[a]	5.90 (4.62)
Smooth surface	4.45 (3.07)[a,b]	4.09 (3.51)	3.62 (2.57)[a]	3.40 (3.25)[b]
	Group D (Control)			
Pit and fissures	5.59 (3.24)	5.35 (3.21)	5.03 (3.19)	5.41 (3.62)
Smooth surface	3.72 (2.60)	3.87 (2.17)	3.88 (1.86)	3.18 (1.77)

Mean (standard deviation). Values sharing the same upper case letter by row and location

TABLE 2 Changes in florescence values according location of WSLs.

Discussion

Modern management of dental caries has three major components: prevention, control and treatment, and is based on appropriate methods of disease detection and diagnosis of pathological changes, including lesion formation at its earliest stages [Ekstrand et al., 2003].

An optimal clinical method for monitoring early caries lesions would permit longitudinal quantification of early mineral loss or gain [Tranaeus et al., 2005]. Non-invasive analysis methods are required for this to be achieved, and laser fluorescence technologies such

	Group A (CPP-ACP) n=51	Group B (CPP-ACFP) n=43	Group C (Fluride varnish) n=68	Group D (Control) n=45
Baseline	21.08 (10.02)[a,b]	22.07 (9.68)[a,b,c]	24.60 (10.17)[a,b,c]	21.18 (10.41)
4 weeks	19.31 (9.34)	14.33 (8.68)[a]	20.68 (9.04)[a]	19.98 (8.74)
8 weeks	13.47 (8.90)[a]	15.98 (9.85)[b]	18.70 (9.06)[b]	18.22 (10.03)
12 weeks	13.02 (8.63)[b]	14.72 (9.49)[c]	16.51 (7.14)[c]	16.33 (11.93)

Mean (standard deviation). Values sharing the same upper case letter by column and group are significantly different

TABLE 3 Evolution by groups of lesion with baseline laser fluorescence value ≥ 10.

as DIAGNOdent represent a promising method for monitoring the progression of caries lesions [Andersson et al., 2007; Mendes et al., 2005; Aljehani et al., 2006; Bader and Shugars, 2004; Anttonen et al., 2004; Francescut and Lussi, 2003].

Regular topical application of fluoride varnish is currently the gold-standard method for protecting teeth against the development of WSLs and for reversing demineralisation. Fluoride varnish has the advantage of being independent of patient compliance and is quick and simple for a dentist to apply. It has been shown to be especially useful in patients undergoing fixed orthodontic treatment [Du M, Cheng et al., 2012].

In the present study, we have performed a 3-month longitudinal study of WSLs with two CPP-ACP-based treatments, compared with the gold-standard treatment of professionally-administered high-concentration fluoride varnish. We have noted that each treatment is to some extent effective, but have also recorded some interesting findings that suggest that each has particular strengths and weaknesses depending on the nature of the lesion. The differences seen between Groups A and B (the CPP groups) and Group C (the varnish group) meant that the null hypothesis was rejected, and the CPP products were concluded to have significantly different actions to fluoride varnish, confirming previous reports [Andersson et al., 2007; Beerens et al., 2010].

The CPP-ACFP treatment (Group B) appeared to reduce caries lesion severity more rapidly than the CCP-ACP treatment (Group A). This increased rate of remineralisation could be caused by the presence of unstabilised calcium, phosphate and fluoride ions in the formulation, because these will readily form fluorapatite in the enamel surface layer. In the absence of CPP, this reaction is undermined by the rapid formation of calcium phosphate phases [Selwitz et al., 2007], but when stabilised by CPP, these ions can diffuse down into the subsurface lesion and form fluorapatite [Reynolds et al., 2004]. Furthermore, some experimental studies have determined that CPP-ACFP solutions may have higher remineralisation capacity than CPP-ACP at acidity levels below pH 5.5 [Cochrane et al., 2008], suggesting that it may continue to function even in highly acidic environments. Our results appear to confirm the superiority of CPP-ACFP to both CPP-ACP and fluoride varnish in the immediate to very short-term (4 weeks) after treatment initiation. Moreover, CPP-ACFP generates a significantly greater decline in caries lesion severity than fluoride varnish after 12 weeks of use, suggesting that daily use of this product may also provide better protection than the current gold standard treatment over the medium term.

In agreement with previous studies [Aljehani et al., 2006; Bailey et al., 2009 Huang et al, 2013], it is likely that clinically visible changes in the WSLs will only be detectable over study periods longer than three months.

When distinguishing between lesions in the pits and fissures and those on smooth surfaces, we found that the effects of all three treatments were minimal, except for significant reductions in fluorescence values in the CPP-ACP and fluoride varnish groups at 8 weeks. Otherwise, values were not significantly different to baseline. Most previous clinical studies conducted to investigate these products have observed only carious lesions on smooth surfaces after orthodontic treatment, and their results are generally inconclusive [Bröchner et al., 2011; Beerens et al., 2010; Aljehani et al., 2006]. However, the one study to differentiate between pit-and-fissure WSLs and those on smooth surfaces found that a CPP-ACP formulation produced significantly higher remineralisation than did a toothpaste containing 1450 ppm fluoride [Altenburger et al., 2010]. We found little evidence for a substantial effect of the fluoride treatments on pit and fissure caries, which may be due to difficulty in penetrating deep into the anatomy of these lesions. This may represent a limitation of all fluoride treatments, and support the recommendation that caries prevention on the occlusal surface is best approached through the early preventive use of fissure sealants where possible [Beauchamp et al., 2009]. However, the lack of effect of the treatments on pits and fissures implies that the effects of each treatment were mostly on smooth surface lesions, a finding confirmed by our data on smooth surface lesions. Therefore, we conclude that all three treatments are effective in remineraliSing WSLs on smooth surfaces, but we are unable to draw conclusions on the superiority [or otherwise] of the CPP compounds compared with the fluoride varnish given the small but significant difference in the baseline value of the varnish group compared with the two CPP groups.

The main limitation of this study is the short study period (three months). We anticipate that further clinical changes in WSLs will become apparent with increased duration of fluoride use, but we were interested mainly in the immediate and short-term effects of these products. Extending the time course further would have introduced even more multiple comparisons and/or reduced the resolution of our short-term characterisation of the effects of the three fluoride treatments. Further study may be required to compare these products over a longer period.

Conclusion

From our analysis, we conclude that the null hypothesis [that there was no significant difference between the three treatments over a three-month period] can be rejected, and that the CPP-ACFP product produces

a larger and more rapid remineralisation of WSLs in 6–14-year-old children in Valencia, Spain. Given that these physiological changes are post-eruptive and represent a biochemical reaction, we predict that these results should also be applicable to other age groups and geographical regions. However, further study is likely to be required to fully characterise the exact magnitude of these short-term effects and to compare the relative outcomes of long-term application of these fluoride treatments.

Conflict of interests

The authors have no conflict of interests to declare.

Author's contributions

CL carried out sample selection, calculation sample size, allocation sequence, assignation to the participants in their groups

AML, data collection, and patient follow up.

LF designed the study, performed data analysis and wrote the draft manuscript.

All authors approved the final manuscript.

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