Effect of CPP-ACP on Remineralisation of Early Caries Lesions in Primary Teeth

B Kargul[1], B Durmus[1], N Bekiroglu[2]

[1]Department of Pediatric Dentistry, Dental School, Marmara University, İstanbul, Turkey, [2]Biostatistics Department, Medical School, Marmara University, Istanbul, Turkey

Abstract

Objective: To evaluate the remineralization effects of the casein phosphopeptide-amorphous calciumphosphate (CPP-ACP) paste on the white spot lesions (WSLs) of the primary teeth thereby to assess its caries-prevention efficacy on early childhood caries (ECC) in vivo. Methods: A total of 11 high caries risk children with 36 noncavitated caries on smooth surfaces in primary incisors and canines were assigned to receive CPP-ACP (GC Tooth Mousse, GC JAPAN) in addition to daily use of fluoridated toothpaste for 4 weeks. Thirty six WSLs on primary incisors and canines were evaluated. During 4 weeks treatment period, all subjects were instructed to use daily fluoridated toothpaste (500 ppm F- as NaF) and additionally applied a CPP-ACP containing paste on respective surfaces for 1 minute, twice a day. Baseline and final mineralization status were determined using a laser-induced infrared fluorescence (FL) device (DIAGNOdent™,KaVoDentalGmbH, Germany). Results: The mean LF results for WSLs at the buccal surfaces of the primary teeth before CPP-ACP paste application was 8.41 ± 12.43 and after was 1.95 ± 4.69 ($P<0.001$). CPP-ACP paste in addition to daily use of fluoridated toothpaste produced an increase in WSLs remineralization of 77% ($P<0.001$). Conclusion: This 4-week clinical study have indicated that twice daily topical applications of CPP-ACP containing paste as an adjunct to a standard oral hygiene programme which includes fluoridated toothpaste, significantly improve the remineralisation of white spot lesions. The usage of CPP-ACP paste with Fluoride toothpaste could be effective for preventing demineralization and promoting remineralization of enamel subsurface lesions.

Key Words: Childhood caries, Fluoridated toothpaste

Introduction

Early childhood caries (ECC), previously known as nursing caries, baby bottle tooth decay is an important public health problem especially in low-income children, with significant negative consequences for the child and the family [1]. The first sign of ECC is denoted by white spot lesions (WSLs) which can be defined as a demineralization of the enamel surface and subsurface, although these lesions can be reversed and do not form cavities [2].

Over the years with clinically proven research, fluoride has been documented to promote remineralization and can be very easily introduced into the oral environment through personal or professional application. However, its ability to remineralize a lesion is dependent on Ca and phosphorus (P) ions and therefore fluoride can be the restrictive factor for net enamel remineralization to occur [3]. Recently, the complex CPP-ACP, derived from a major protein found in milk called casein is presented as an alternative remineralizing agent that is remarkably capable of stabilizing calcium phosphate, look promising as adjunctive treatments to topical fluorides in the non-invasive management of early caries lesions [4,5]. Stabilized calcium phosphate, maintaining a state of supersaturation of these ions in the oral environment when delivered in a mouth rinse, tooth pastes, sealants, varnishes, chewing gums and promotes the remineralization of enamel subsurface lesions *in vitro* and *in situ* [6-10].

Conventional methods for caries detection are not capable of quantifying the mineral loss or gain occurring as a result of demineralization and remineralization processes, respectively [11]. Besides WSLs are not detectable visually until they have progressed 200-300 μm into the enamel. In this sense, quantitative methods have been developed for caries detection and for monitoring changes in the mineral content [12]. Some of these methods are based on the fluorescence phenomenon emitted by bacterial porphyrins (fluorophores), molecules that are excited by a light source with a specific excitation wavelength [13,14].

On visual examination, well-defined clinical criteria are the methods of first choice, moreover when examining free smooth surfaces *in vivo*, since the clinician's ability has been pointed out as a valid method to determine caries activity [15]. However, it is still difficult to make a decision on subclinical caries diagnosis. A diagnostic system could have the advantage of objectively recording noncavitated stages of caries lesions [16].

A laser-based diagnostic system (LF), DIAGNOdent (KaVo, Biberach, Germany) (DD) was developed using a diode laser as a light source and a photodiode combined with a long-pass filter as detector for the determining and quantification of caries lesions in clinical situations [17]. Importance of early detection of caries activity is inevitable especially at incipient lesions.

Although several *in vitro* and *in situ* studies [6-10] have proved the remineralizing potential and success rate of products based on agents containing CPP-amorphous calcium phosphate (CPP-ACP) with fluoride or without fluoride, very few exist *in vivo* on efficacy of CPP-ACP at WSLs on primary teeth. Therefore, this *in vivo* study was designed to evaluate the remineralization potentials of topically applied CPP- ACP (ToothMousse, GC) on the early enamel lesion of the children with childhood caries (ECC).

Corresponding author: Betul Kargul, Dentistry Faculty, Department of Pediatric Dentistry, Marmara University, Başıbüyük Yolu 9/3 34854 Başıbüyük/Maltepe/Istanbul, Tel: 009002164211621; E-mail: bkargul@marmara.edu.tr

Methods

The procedures of the study followed the ethical standards of the Marmara University ethical committee on human experimentation and with the Helsinki Declaration.

Sample selection

This study was conducted 6 children who were treated (4 boy 2 girl; between 3-4.5 years of age; mean age ± SD: 3.95 ± 0.45) in Marmara University, Dental School, Department of Pediatric Dentistry, Istanbul, Turkey. All subjects provided informed written consents.

Assessment criteria

The clinical selections were carried out in clinic under reflector light conditions using sterile mirrors and blunt probes with compressed air. The teeth were cleaned and plaque was removed. Thirty six non cavitated caries on free smooth surfaces in primary incisors and canines were chosen for evaluation. WSLs were categorised according to the International Caries Detection and Assessment System (ICDAS II; grades 0-3).

After visual examination, Laser fluorescence (LF) measurements DIAGNOdent®(KaVoDentalGmbH, Germany) was conducted and values were recorded as baseline data. LF provides an indirect measure of the extent of demineralisation of noncavitated caries: the tooth surface is exposed to light of wavelength 655 nm which causes organic matter in the demineralised lesion to fluoresce. The intensity of the fluorescence is converted into an arbitrary numerical scale by the instrument. Following the manufacturer's instructions, calibration of the DD was performed with a ceramic standard provided by the company. A DD with a short cylindrical tip B measured each demineralized smooth labial surfaces after the surface was washed and air-dried. A set of DD readings was taken; if peak values differed between readings, the numbers were averaged to determine the surface's DD reading.

Procedures

During 4 weeks treatment period, all children were instructed to brush their teeth after breakfast and before bedtime with a pea-sized quantity of fluoride toothpaste (500 ppm F- as NaF) and they were required to apply the CPP-ACP containing paste (GC Tooth Mousse, GC JAPAN, Japan) to the WSLs with a finger for 1 minutes, twice a day after their morning and evening oral care regimes and to avoid eating or drinking for at least thirty minutes after the application.

All the surfaces were examined using DIAGNOdent®(KaVoDentalGmbH, Germany) to assess for any numeric change after the remineralization procedure.

Statistical analysis

All data were processed by the SPSS 15.0 software package. The baseline and after treatment LF data were subjected to Paired t-test and $p \leq 0.05$ was considered to be significant.

Results

The value of 9 is determined as the cut-off point and the value of samples showing above this number is considered as the presence of surface lesion on the tooth surface. All of the WSLs were either stabilized or regressed during the study. The mean and standard devaition of LF results for WSLs at the buccal surfaces of the primary teeth before CPP-ACP paste application was 8.41 ± 12.43 and after was 1.95 ± 4.69 LF values were reduced at 4 weeks. The lesion regression of treatment regime was found to be significant at P<0.001 (*Table 1*).

Table 1. *Mean laser fluorescence readings (mean±standard deviation) in WSLs for treated teeth at baseline and after 4 weeks CPP-ACP containing paste usage.*

Tooth	Before CPP-ACP (Mean ± SD)	After CPP-ACP (Mean ± SD)	P
63	9.11 ± 14.23	3.98 ± 8.37	0.008
62	14.56 ± 16.07	2.97 ± 5.78	0.005
61	8.62 ± 11.99	1.77 ± 3.26	0.005
51	4.30 ± 5.44	0.17 ± 0.58	0.008
52	8.12 ± 15.10	1.36 ± 3.12	0.012
53	5.74 ± 8.11	1.45 ± 2.74	0.139

CPP-ACP containing paste with oral hygiene daily routine produced an increase in WSLs remineralization of 77%.

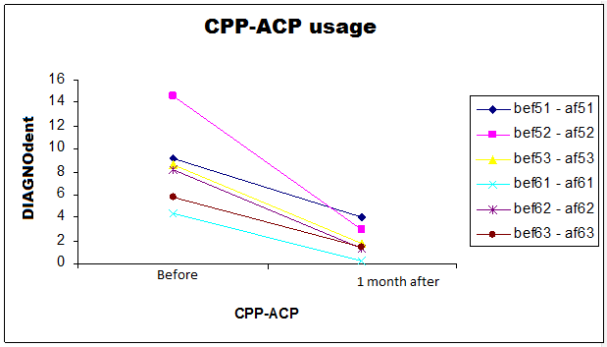

Figure 1. *Mean decrease in laser fluorescence values after 4 weeks usage of CPP-ACP containing paste.*

The difference of the mean LF values between the baseline and after treatment regime was statistically significant (P<0.005) (*Figure 1*) in all teeth groups except #53. The maximum remineralization was seen in teeth number #62 followed by #52 and then #61. According to the mean reductions in LF measurements between baseline and after treatment, significant improvements in the remineralisation of the lesions were observed for all teeth groups except number 53 after the 4-week period (P>0.005).

Discussion

Although saliva has a remineralization potential, remineralization produced by saliva is minimal and it occurs on the surface layer of the lesion only and new remineralizing

systems would be needed to achieve more pronounced lesion regression [18,19]. In order to perform mineral deposition within the body of the lesion, first calcium and phosphate ions must penetrate into the surface layer of enamel. This explains why the CPP-supported metastable calcium phosphate solutions are such efficient that in remineralizing solutions. Because they are able to consume the acid generated during enamel lesion, so that by remineralization it is generated more calcium and phosphate ions, including CaHPO4, thus maintained the high concentration gradient into the lesion [20]. Based on the evidence above, we observed that the use of twice daily topical applications of CPP-ACP paste with fluoridated toothpaste significantly improved the remineralisation of the WSLs. The advantage of using CPP–ACP complexed with fluoride is the simultaneous availability of calcium, phosphate, and fluoride [21].

Alhough the CPP- ACP revealed its efficacy in caries prevention in most studies, whether it has added promoting effect or not is still controversial. Yimcharoen et al. [22] investigated CPP-containing toothpaste and compared it with fluoride-containing toothpastes on remineralization of caries-like lesions in primary teeth enamel, using polarized light microscopy. They concluded that CPP-containing toothpaste, 260 ppm fluoride-containing toothpaste and a 500 ppm fluoride-containing toothpaste all had significant efficacy for inhibiting demineralization of carious lesions; however, 500 ppm fluoride-containing toothpaste inhibited lesion progression better than CPP-containing toothpaste and a 260 ppm fluoride-containing toothpaste. This result may due to the fact that The effectiveness of the CPP-ACP paste can be enhanced in the oral cavity if a biofilm exists, because it can bind to CPP and act as a reservoir for the calcium and phosphate ions [22].

Altenburger et al. [23] evaluated the effect of casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP), as an adjunct to average oral hygiene, on the fluorescence of initial carious fissures and pits *in vivo*. Fissures additionally treated with the CPP-ACP containing cream showed significantly lower laser fluorescence values after Day 15 ($P=0.001$) and Day 22 ($P<0.001$) compared to the control group [23]. These findings were in accordance with the our results which showed a significant improvement in early carious lesions regression on primary teeth by the daily use of CPP-ACP-containing cream adjunct with flüoride brushing at 1 month.

Moreover, the results of our study support previous reports by Akin and Başciftci [24] and Andersson et al. [25] who studied the effects of pastes containing CPP-ACP, respectively, on white spot lesions of postorthodontic treatment. Akin and Basciftci [24] compared the effects of sodium fluoride mouth rinse, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)in treating white spot lesions. All participants were instructed to use 20 ml of neutral 0.025% sodium fluoride rinse, only the participants in the CPP-ACP group were instructed to use tooth mousse twice a day in addition to fluoride toothpaste for 6 months. The area of the white spot lesions decreased significantly in all groups [24]. Andersson et al. [25] observed that daily topical application of a dental crème containing CPP-ACP for 3 months followed by a 3-month period of daily tooth brushing with fluoridated toothpaste helped in the complete elimination of the post-orthodontic WSLs .

Llena et al. observed that 4-week use of CPPACFP was superior to duraphate fluoride varnish in remineralizing smooth surface WSLs [26]. However, Beerns et al. [27] observed that the use of CPP-ACFP crème for 12 weeks had no clinical advantage over normal hygiene in the remineralization of WSLs.

In this study the procedure was daily hone routime of CPP-ACP paste application performed by parents after toothbrushing with 500 ppm fluoridated toothpaste as we expected the synergistic effect of CPP-ACP and fluoride in remineralization of the lesion. After 1 month, we found the number of lesions that experienced regression was 77%. Although we had limited chance to be able to put into practice regarding the sample size, we observed that he usage of CPP-ACP paste with fluoride toothpaste could be effective for preventing demineralization and promoting remineralization of enamel subsurface lesions.

Conclusion

This study showed that preventive intervention methods play the major role in arresting WSL and dental caries in young children. Oral hygiene instruction alone was not sufficient enough to reduce WSL. However, oral hygiene instruction together with the application CPP-ACP was an effective method to reduce WSLs mineral loss.

Nevertheless, further study might be conducted for a longer follow-up time, other application times or absence of fluoridated toothpaste to confirm these initial results *in vivo*.

References

1. Milnes AR. Description and epidemiology of nursing caries. *Journal of Public Health Dentistry.* 1996; **56**: 38-50.

2. Paula AB, Fernandes AR, Coelho AS, Marto CM, Ferreira MM, et al. Therapies for White Spot Lesions-A Systematic Review. *Journal of Evidence-Based Dental Practice.* 2017; **17**: 23-38.

3. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. *Caries Research.* 2008; **42**: 88-97.

4. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? *Australian Dental Journal.* 2008; **53**: 268-273

5. Lata S, Varghese NO, Varughese JM. Remineralization potential of fluoride and amorphous calcium phosphate-casein phosphopeptide on enamel lesions: an *in vitro* comparative evaluation. *Journal of Conservative Dentistry.* 2010; **13**: 42-46.

6. Kargul B, Altinok B, Welbury R. The effect of Casein Phosphopeptide-Amorphous Calcium Phosphate on Enamel Surface Rehardening:An *in vitro* study. *European Journal of Paediatric Dentistry.* 2012; **13**: 123-127.

7. Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC. Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. *The Journal of Biological Chemistry.* 2005; **280**: 15362-15369.

8. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. *Special Care in Dentistry.* 1998; **18**: 8-16.

9. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium

in a mouthrinse or sugar-free chewing gum. *Journal of Dental Research.* 2003; **82**: 206-211.

10. El Mehdi H, Hind R, Hakima C. The benefits of casein phosphopeptid-amorphous calcium phosphate (CPP-ACP) in pediatric dentistry. *Scholars journal of dental sciences.* 2016; **3**: 247-250.

11. Spiguel MH, Tovo MF, Kramer PF, Franco KS, Alves KM, Delbem AC. Evaluation of laser fluorescence in the monitoring of the initial stage of the de-/remineralization process: an *in vitro* and in situ study. *Caries Research*. 2009; **43**: 302-307.

12. Maupomé G, Pretty IA. A closer look at diagnosis in clinical dental practice: part 4. Effectiveness of nonradiographic diagnostic procedures and devices in dental practice. *Journal of Canadian Dental Association.* 2004; **70**: 470-474.

13. Hibst R, Paulus R, Lussi A. A detection of occlusal caries by laser fluorescence: basic and clinical investigations. *Medical Laser Application.* 2001; **16**: 295-313.

14. Bader JD, Shugars DA. A systematic review of the performance of a laser fluorescence device for detecting caries. *The Journal of the American Dental Association.* 2004; **135**: 1414-1426.

15. Alanen P, Hurskainen K, Isokangas P, PietiläI, Levänen J, et al. Clinician's ability to identify caries risk subjects. *Community Dentistry and Oral Epidemiology.* 1994; **22**: 86-89.

16. Pinellia C, Serrab MC, Loffredo LCM. Validity and reproducibility of a laser fluorescence system for detecting the activity of white-spot lesions on free smooth surfaces *in vivo*. *Caries Research.* 2002; **36**: 19-24

17. Al-Khateeb S, Forsberg C M, de Josselin de Jong E, Angmar-Mansson B. A longitudinal laser fluorescence study of white spot lesions in orthodontic patients. *American Journal of Orthodontics and Dentofacial Orthopedics.* 1998; **113**: 595-602.

18. Grewal N, Gumber S, Kaur N. Comparative evaluation of enamel remineralization potential of processed cheese, calcium phosphate-based synthetic agent, and a fluoride-containing toothpaste: An in situ study. *Journal of Indian Society of Pedodontics and Preventive Dentistry.* 2017; 35: 19-27.

19. Amaechi BT, Higham SM. In vitro remineralisation of eroded enamel lesions by saliva. *Journal of Dentistry.* 2001; 29: 371-376.

20. Hegde AM, Naik N, Kumari S. Comparison of salivary calcium, phosphate and alkaline phosphatase levels in children with early childhood caries after administration of milk, cheese and GC tooth mousse: an in vivo study. *The Journal of clinical pediatric dentistry.* 2014; 38: 318-325.

21. Chandak S, Bhondey A, Bhardwaj A, Pimpale J, Chandwani M. Comparative evaluation of the efficacy of fluoride varnish and casein phosphopeptide – Amorphous calcium phosphate in reducing Streptococcus mutans counts in dental plaque of children: An in vivo study. *Journal of International Society of Preventive and Community Dentistry.* 2016; 6: 423-429.

22. YimcharoenV, Rirattanapong P, Kiatchallermwong W. The effect of casein phosphopeptide toothpaste versus fluoride toothpaste on remineralization of primary teeth enamel. *The Southeast Asian Journal of Tropical Medicine and Public Health.* 2011; 42:1032-1040.

23. Altenburger MJ,Gmeiner B, Hellwig E, Wrbas KT, Schirrmeister JF. The evaluation of fluorescence changes after application of casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP) on early carious lesions. *American Journal of Dentistry.* 2010; 23: 188-192.

24. Akin M, Basciftci FA. Can white spot lesions be treated effectively? *The Angle Orthodontist.* 2012; 82: 770-775.

25. Andersson A, Sköld-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. *Oral Health & Preventive Dentistry.* 2007; 5: 229-233.

26. Llena C, Leyda AM, Forner L. CPP-ACP and CPP-ACFP versus fluoride varnish in remineralisation of early caries lesions. A prospective study. *European Journal of Paediatric Dentistry.* 2015; 16: 181-186.

27. Beerens MW, van der Veen MH, van Beek H, ten Cate JM. Effects of casein phosphopeptide-amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up. *European Journal of Oral Sciences.* 2010; 118: 610–617.