Comparative Evaluation of Casein Phosphopeptide - Amorphous Calcium Phosphate and Fluoride in Managing Early Caries Lesions

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The aim of the present study was to assess the remineralizing potential of 10% casein phosphopeptide-amorphous calcium phosphate complex (CPP-ACP) -Recaldent used alone or with fluoride (CPP-ACFP) as compared to fluoride mouthrinse for the non-invasive treatment of occlusal non-cavitary caries lesions in vivo.A total of eighty subjects were randomly assigned to four groups, i.e. group 1 - 10% CPP-ACP, group 2- 10% CPP-ACP + 0.2% NaF, group 3- 0.05% NaF mouthrinse and group 4 –control. The lesions were assessed using visual examination and DIAGNOdent measurements. After 12 weeks of using the corresponding product, the results showed that the highest decrease in fluorescence was produced by CPP-ACFP (from 16.28±1.40 to 6.53±0.59, p=0.002), followed by CPP-ACP (from 15.70±1.82 to 8.18±0.70, p=0.004) and fluoride mouthrinse (from 14.32±1.72 to 7.88±0.68, p=0.005), significantly higher than in the control group (p<0.05). No significant differences were found between the three treatment groups.

Keywords: CPP-ACP, CPP-ACFP, fluoride mouthrinse, remineralization.

Dental caries is defined as the destruction of tooth tissue by the action of oral micro-organisms. The first step of the process involves demineralisation of tooth hard tissue by organic acids produced from fermentable carbohydrates by cariogenic bacteria located in dental plaque [1, 2]. However, dental caries is not simply a continual cumulative loss of tooth minerals, but rather a dynamic process characterized by alternating periods of demineralization and remineralization. Remineralization can occur as a natural repair process based on salivary minerals, but this is a slow and sometimes ineffective process. The principles of minimally invasive dentistry clearly dictate the need for clinically effective measures to remineralize early enamel caries lesions [3-5], and remineralization is a major advance in the clinical management of the disease.Fluoride ions promote the formation of fluorapatitein enamel in the presence of calcium and phosphate ions produced during enamel demineralization by plaque bacterial organic acids. This is now believed to be the major mechanism of fluoride ion's action in preventing enamel demineralization. Fluoride ions can also drive the remineralization of previously demineralized enamel if enough salivary or plaque calcium and phosphate ions are available when the fluoride is applied. However, for every two fluoride ions, tencalcium ions and six phosphate ions are required to form one unit cell of fluorapatite (Ca10(PO4)6F2). This means that, in case of topical application of fluoride, the availability of calcium and phosphate ions can be the limiting factor for net enamel remineralization to occur [1, 6, 7].

Fluoride formulations include mouthwashes, toothpastes, gels and varnishes, and the pivotal discovery of fluoride as agent that could prevent dental caries was one of the most important landmarks in dentistry [3]. As the concentration and bioavailability of calcium ions are the limiting factors in the remineralization process, a number of home-use and clinical products have been developed to enhance the calcium and phosphate concentrations of saliva and plaque [8, 9]. These include dentifrices, chewing gums and topical pastes [10]. Bioactive agents based on milk proteincasein phosphopeptide (CPP) with amorphous calcium phosphate (ACP) have been also developed to release elements that enhance remineralization of the enamel and dentin [11]. CPP-ACP, a milk-derivative, comprises peptide fragments that are rich in phosphorylated seryl and glutamic acid residues that bind to amorphous calcium phosphate nanoparticles. The peptide residues stabilise the amorphous calcium phosphate phase and inhibit its premature crystallisation in the oral cavity, thus maintaining a supplyof bioavailable calcium and phosphate ions for subsurface remineralisation. These CPP-ACP nanoclusters are reported to adhere to enamel, plaque and pellicle, to inhibit bacterial adhesion to the tooth, and to act as a pH buffer against acid assault [10, 12].When CPP ACP is combined with fluoride, the fluoride ions react with CPP ACP to form CPP amorphous calcium fluoride phosphate (ACFP). The CPP ACFPnanocomplex provides calcium, phosphate and fluoride ions to the surface of the teeth and therefore has an important effect on enamel remineralization[11].

The aim of the present study wasto evaluate the efficacyof 10% casein phosphopeptide-amorphous calcium phosphate complex (CPP-ACP) -Recaldentused alone or with fluoride (CPP-ACFP) as compared to fluoride mouthrinse for the remineralization of occlusal non-cavitary caries lesions *in vivo.*

Experimental part

Study population

A randomized controlled trial was conducted on 80 students (37 female and 43 male) attending the Faculty of Dental Medicine of the Grigore T. Popa University of Medicine and Pharmacy in Iasi, Romania. The inclusion criteria for the study were: adults with good general health and at least one occlusal surface with incipient carious lesions in the pits and fissures. Exclusion criteria were: subjects with systemic diseases, who were sensitive to milk proteins, presence of orthodontic treatment,prolonged use of medications affecting the salivary flow, developmental defects,multiple occlusal and proximal

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restorations, dentalfluorosis. Filled and decayed teeth were excluded.

Informed consent was obtained from the subjects prior to the study. Dataprivacy as well as the impossible identification ofsubjectsin the case the results are published were ensured [13].

Examinations were performed by 2 calibrated dentists. The Kappa index had values ranging between 0.80 and 0.86.

Visual examination and DIAGNOdent measurements

Oral examination of the subjects was performedunder a standard dental operatory light, using mouth mirror and dental probe. Professionaloral prophylaxis was performed for all subjectsusing an ultrasonic scaler and prophylaxis paste, thenthe teeth were thoroughly rinsed with water. Thefissures were cleaned with a WHO probe to removeany remaining plaque or debris, then the occlusal surfaces were cleaned with an alcohol swab andlater dried using the air-spray of the dental unit for 10s. Areas of demineralization were identified basedon the presence of a white opaque area followingre-wetting and drying. The occlusal surfaces were scoredusing Full ICDAS criteria [14]. Tooth surfaces classified 1 and2 were includedin the study.

Because clinical detection of early carious lesionsis a challenge, several detection systemsbased on laser/light fluorescence are currently beingused as an adjunct to visual detection. DIAGNOdent(Kavo; Biberach, Germany) is a caries detectionsystem based on laser-stimulated fluorescence.It has proven to be more sensitive than visualexamination and bite-wing radiographs in detectingearly non-cavitated occlusal enamel lesions [15]. A further advantage is that DIAGNOdentcan quantify the degree of demineralization by digitalreadings and these readings are reproducible [16].

The original DIAGNOdent consists of two probes, Aand B, used for occlusal and proximal surfaces, respectively.In this study, probe A was used to recordthe demineralization values. The instrument wascalibrated for each patient on a ceramic mount provided by the producer.The teethwere isolated with sterile cotton rolls and the surfacesof the teeth were dried with an air spray.Thefluorescence of a sound spot on the facial surface ofthe tooth was measured to provide a baseline value;this value was then subtracted electronically from thefluorescence of the site to be measured. Finally, the tip of the laser device was placed on the demineralized areaand rotated around a vertical axis until the highestfluorescence reading was obtained.Three readings for thesame site were taken and the peak reading displayed on the panel of the DIAGNOdent during the scan was recorded for each tooth surface. Based on producer's recommendations and the findings of previous studies [15], fissures with DIAGNOdent scores between 11 and 30 were included in the present study.

Treatment procedures

Subjects identified with tooth demineralisation were randomly and equally (n=20) allocated to one of the following four groups: group 1-10% CPP-ACP (Tooth Mousse, GC; Tokyo, Japan) – the patients were advised to apply the topical creme 2 times/day on the occlusal surfaces, after brushing teeth; a minimum of a pea-sized amount was recommended for each dental arch, left undisturbed on the teeth for min , after which eating and drinking were prohibited for 30 min; group 2 -10% CPP-ACP + 0.2% NaF (Tooth Mousse Plus, GC; Tokyo, Japan) – the same manner of application; group 3 -0.05% NaF mouthrinse (Colgate Plax) - 20 mL, for 30 s, 2 times/day after toothbrushing; group 4 - control. All groups were provided with oral hygiene instructions and a package of dental products for home-use. All patients were instructed to brush twi-ce daily (morning and evening) using a soft-texture toothbrush and fluoridated toothpaste (blend-a-med, 1450 ppm F). The patients were also advised to avoid any supplementary fluoridated products and prevent from eating too much sugar and acidic food or drink.

The lesions were evaluated at the start of the study and at 4, 8 and 12 weeks. For DIAGNOdent readings, declining values representedan improvement while increasing values representeddeterioration of carious lesions.

Statistical analysis

The data were analysed using SPSS statistical software package forwindows version 18.0. Paired sampled t-tests were performed to compare the changes inremineralisation between baseline and post-treatment measurements within the same group. One way ANOVA was used to determine significant differences between the four groups.The confidenceinterval was 95% and the significance was set at a level of $p< 0.05$.

Results and discussions

A total of 80 subjects of age range 21 to 26 years were recruited into the study and none was excluded throughout the 12 weeks period of evaluation. The number of occlusal surfaces examined in each group was comparable: group 1 (n=43), group 2 (n=46), group 3 (n=40), group 4 (n=41).

The dynamic of ICDAS scores from baseline during the 12 weeks of examination is presented in table 1. The change was evaluated as decrease, increase or no change (stagnation). The highest percentage of teeth with a decrease in the ICDAS score was found in group 2 (CPP-ACFP, 6.2%), at the 12 weeks examination, while the highest percentage of teeth with an increase in the ICDAS score was found in group 4 (control, 18.4%), at the same examination. However, the only statistically significant differences were found between the treatment and the control groups, in what concerns the percentage of teeth with an increase in ICDAS score, during the three recall examinations ($p<0.05$).

Comparison of DIAGNOdent values evaluated beforeand after the application of treatment showed a decrease in fluorescence, suggesting an increase in lesions mineralization, significant in groups 1, 2 and 3 and not significant in group 4 (control), at the comparison between baseline and final examination (table 2). The highest decrease in fluorescence was found in group 2 (CPP-ACFP, p=0.002), where the difference between T_0 and T_3 examination was 9.75(meaning a reduction of 60% of the baseline value), followed by group 1 (CPP-ACP, p=0.004), with a decrease of 7.52 or 48% of the baseline value and group 3 (NaF mouthrinse, p=0.005), with a decrease of 6.44 or 45% of the baseline value. Inter-group comparison showed significant differences only for the T_2 and T_3 examination, between the treatment and control groups (p=0.012 and p=0.005, respectively). No significant differences were found between the three treatment groups.

The difference between visual examination and DIAGNOdent readings in what concerns the evolution of lesions may be explained by the fact that, in case of early caries in pits and fissures, remineralization is not followed by a return of the lesion to the initial aspect of sound enamel. Therefore, although the laser-fluorescence evaluation proved a significant increase in the mineral content of the lesions in the three treatment groups until the end of the

Table 1

CHANGE FROM BASELINE ICDAS SCORE, BY TREATMENT GROUP

Time interval	Change in ICDAS scores from baseline	Study group(% teeth)				*p* value
		Group 1	Group 2	Group 3	Group 4	
T_1 (4 weeks)	*Decrease*	2.4	3.6	1.8	0.0	0.062
	No change	94.9	94.2	95.3	94.9	0.956
	Increase	2.7	2.2	2.9	5.1	0.036*
T_2 (8 weeks)	*Decrease*	4.2	5.2	4.4	2.6	0.070
	No change	92.3	91.7	91.4	88.9	0.630
	Increase	3.5	3.1	4.2	8.5	0.009*
T_3 (12 weeks)	*Decrease*	5.1	6.2	4.8	4.5	0.095
	No change	86.1	86.2	86.0	77.1	0.540
	Increase	8.8	7.6	9.2	18.4	0.012*

*=statistically significant

Table 2

DESCRIPTION AND COMPARISON OF DIAGNODENT SCORES AT VARIOUS TIME INTERVALS OF OBSERVATION AMONG SUBJECTS OF 4 GROUPS

Study group	DIAGNOdent scores (mean ± SD)				Significance (*p* value, t test) between T_0 and T_3
	T_0 (baseline)	T_1 (4 weeks)	T_2 (8 weeks)	T_3 (12 weeks)	
Group 1	15.70±1.82	11.68±1.80	9.52±0.92	8.18±0.70	0.004*
Group 2	16.28±1.40	11.25±1.36	8.21±0.86	6.53±0.59	0.002*
Group 3	14.32±1.72	12.16±1.55	9.13±0.91	7.88±0.68	0.005*
Group 4	15.18±1.60	14.82±1.46	14.12±1.15	13.25±0.97	0.070
p value, inter-group	0.932 NS	0.070 NS	0.012*	0.005*	

*=statistically significant

study, the decrease in ICDAS scores of the same groups was not significant.

The optimal efficacy oftopical fluoride therapy for enamel remineralization of incipient carious lesions and reduction of enamel solubility is generally recognized[17]. Fluoride agents that release a high dose offluoride initially (burst effect) are more effective for increasing enamel resistance against decalcification. A low concentration of fluoride is more effective in enamel remineralization. The high dose of fluoride physically blocks the surface layer of enamel to penetration of calcium ions to subsurface layers. Thus, high dose of fluoride is recommended in inhibiting lesion formation and low dose of fluoride for the remineralization and controlling lesion progression [18].

CPP-ACP is currently marketed under the brand name Tooth Mousse. It can stop the progression of caries since it decreases demineralization and enhances remineralization.The synergistic effect of CPP-ACP and fluoride in reducing early carious lesions is due to the formation of CPP-stabilized amorphous calcium fluoride phosphate, resulting in increased concentration of bioavailable calcium and phosphate ions [19]. The CPP-bound ACP acts as a reservoir of calcium phosphate ions, including the neutral ion pair CaHPO4 which is formed in the presence of acid [1, 20]. When acid is formed by the plaque bacteria, the CPP-bound ACP buffers the plaque pH, and in doing so, it dissociates to calcium and phosphate ions includingCaHPO4. The increased plaque calcium and phosphate ions offset any fall in pH, thereby preventing enamel demineralization. In the presence of fluorides, formation of CPP-ACFP nano-complexes takes place, and when the pH falls, breakage of the nano-complex leads to formation of calcium ions, phosphate ions, and neutral species CaHPO4 and HF. These ions following concentration gradient move inside the subsurface lesion, thus leading to formation of fluorapatite [18, 21, 22].The resultingsurface would probably be much more caries-resistant compared to theoriginal enamel, but it still retains a demineralized appearance [23].

The results of the present study are in line with those of other authors, who found that the use of CPP-ACP combined with fluoride produced a better remineralization than the use of fluoride alone [16, 10], although no significant differenceswere observed. A meta-analysis conducted in 2018 concluded that fluorides combined with CPP-ACP treatment produce significantly better efficacyfor occlusal early caries lesionsthan fluorides alone [24].

At the same time, the use of 1450 ppm of fluoridetoothpaste twice daily alone had some beneficial effect on the regression of early carious lesions, although not statistically significant.

The remarkable discrepancies in the literature regardingthe clinical relevance of CPP-ACP have been attributedto variations in study-design, duration of clinical trials, differences in the activity and severity of lesions, and thepossible pathological dissimilarities between various types of lesions. The development of CPP-ACPtechnology is relatively recent, and it is currentlyacknowledged that further clinical studies are required beforedefinitive recommendations for its use can be made [10].

Conclusions

The findings of this 12-week clinical study indicated thatCPP-ACP, CPP-ACFP and fluoride mouthrinse all

produced remineralization of early carious lesions, significantly higher than that in the control group. The highest remineralization was produced by CPP-ACFP.

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