Effect of Casein Phosphopeptide–Amorphous Calcium Phosphate as a Remineralizing Agent – An In Vivo Study

Abstract

Introduction: Demineralization and remineralization have a crucial impact on the hardness and strength of teeth. Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) Trident white chewing gum has anticariogenic property and also stimulates saliva, which buffers the oral cavity and promotes remineralization. Trident sugar-free gum, therefore, is an excellent delivery vehicle for promoting enamel remineralization. Aims and Objectives: The aim of this study is to show that CPP-ACP-containing chewing gum would increase the level of calcium concentration of saliva, thereby supplying calcium and phosphate to whole dentition for a prolonged period and aid in remineralization of tooth surfaces. Materials and Methods: An in vivo nonrandomized clinical trial study was carried among 60 children. Unstimulated saliva from each 60 selected participants was collected. Then each participant was given two pellets of chewing gum containing CPP-ACP and asked to chew for a period of 20 min, after which saliva samples were again collected from each participant. The study was carried out for 15 days, and at three intervals, calcium and phosphorus levels were assessed using affiliated reagent kits and spectrophotometer. Results: Significant difference was found in the calcium and phosphorus concentration of saliva before and after chewing CPP-ACP-containing chewing gum. When post calcium and phosphate levels were analyzed among different time intervals, a highly statistically significant difference was observed (P = 0.000). Conclusion: Clinical trial study shows that chewing sugar-free gum containing CPP-ACP can be regarded as an additional caries prevention tool.

Keywords: Amorphous calcium phosphate, casein phosphopeptide, enamel demineralization, enamel remineralization, nano hydroxyapatite

Introduction

Teeth are tools that have evolved to aid in the survival of the species. Nature has fashioned the hard enamel covering to serve this function. It is a paradox that though enamel is the hardest biological substance, its acellular and avascular nature makes it incapable of any natural defense mechanism based on cellularity. When tooth erupts into the oral cavity, the hypomineralized enamel encounters a highly complex ecology. Imbalance in this complex environment holds the clue to the origin of this unique disease called dental caries. The onset of dental caries requires the establishment of necessary conditions for mineral dissolution.[1]

Tooth enamel is the highest mineralized tissue of the body. It is mainly composed of 96% inorganic material, 4% organic material and water. Oral environment is a combat zone for activities such as demineralization and remineralization. Therefore, it is necessary to maintain equilibrium between these two processes.[2] Nowadays, newer noninvasive interventions for noncarious lesions are implicated as a preventive treatment.[3] Casein, a bovine milk phosphorus protein, is a natural food component found in cow’s milk, which interacts with calcium and phosphate ions. Technically, it is known as casein phosphopeptide–amorphous calcium phosphate (CPP-ACP). It contains cluster sequence of [−Ser(P)−Ser(P)−Ser(P)−Glu−Glu] which stabilizes calcium and phosphate in solution and increases the level of calcium and phosphate in dental plaque and tooth. A pioneer of CPP-ACP in dentistry is Professor Eric Reynolds from the School of Dental Science at the University of Melbourne in Australia.[4]

Remineralization can be enhanced by enriching tissues with hydroxyapatite.
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which is the same inorganic component as enamel. ACPs are highly soluble and can be readily converted to hydroxyapatite which makes it a suitable remineralizing agent.

The most common mode of using CPP-ACP is through sugar-free sorbitol or xylitol-based chewing gums. Apart from chewing gum, the other vehicles are milk, mouth rinses, lozenges, and dental cream. Trident is a commercially available (Trident Total; Kraft Foods/Cadbury Adams SP, Brazil) chewing gum containing CPP-ACP and patented as Recaldent.

Therefore, this nonrandomized clinical trial study was done to assess the efficacy of CPP-ACP as a remineralizing agent.

**Materials and Methods**

An in vivo nonrandomized clinical trial study was conducted. For this proposed study, 60 children age between 8 and 14 years were selected. This study was reviewed by the institutional ethical committee and ethical approval was obtained. Informed consent was then obtained from the caretakers of the participants. The inclusion criteria were as follows: children with no active carious lesion, no pathological changes in oral mucosa or periodontal tissue, sample collection done in morning hours, and in those who had breakfast 2 h before (children not consumed food or drink between this period). Exclusion criteria were as follows, children who were allergic to milk protein, children who were on antibiotics as it may hamper salivary flow, and children with fluorosis.

The following armamentarium was used for the study: CPP-ACP-containing chewing gum (Trident Cadbury Adams USA) watermelon flavor serving two pellets – 3 g, serum calcium and serum phosphorus reagent kit (Accucare Labcare Diagnostic Pvt., India), spectrophotometer, sputum bottle, and micropipette. The protocol of the study was explained to participants in detail, and it was informed that the study will be carried out in three phase – baseline, first week, and second week. Before the study, 1.5 mL of saliva sample was collected and labeled as BEFORE SAMPLE. Analysis of saliva sample was started to detect presalivary calcium and pre-salivary phosphorus level with spectrophotometer. Meanwhile, the subjects were given two pellets of CPP-ACP “Trident chewing gum” (Cadbury Adams USA) and asked to chew for 20 min, and again 1.5 mL of saliva sample was collected and labeled as AFTER SAMPLE. Immediately after collection, salivary calcium and phosphorus levels were detected with spectrophotometer.

Then for the next six consecutive days, the subjects were given two pellets of chewing gum [Figure 4], and on the seventh day again pre- and post-salivary calcium and phosphorus levels were detected with the help of spectrophotometer and the same procedure was again repeated for the next six days, and then on 15th day again pre- and post-salivary calcium and phosphorus levels were detected. The data were statistically analyzed using
Results

In this study, a total of 60 participants with a mean age of 9.35 ± 1.02 years were selected. Before chewing trident gum, the mean pre-calcium level in baseline was found to be 9.68 ± 1.13 mg/dL and pre-phosphorus level in baseline was 4.67 ± 0.25 mg/dL. After chewing the gum containing calcium phosphate, it resulted in an increase in the mean salivary calcium level to 10.48 ± 0.98 mg/dL and phosphorus level to 4.80 ± 0.11 mg/dL [Table 1]. Similar results were obtained in the first and second consecutive weeks with a mean pre-salivary calcium level of 9.80 ± 1.14 and 9.31 ± 1.37 mg/dL, respectively, and salivary phosphorus level of 4.76 ± 0.18 and 4.73 ± 0.22 mg/dL, respectively. The chewing gum in the consecutive week resulted in increased post-salivary calcium levels to 10.78 ± 1.10 and 11.47 ± 1.04 mg/dL, respectively, and post-salivary phosphorus levels to 5.32 ± 0.36 and 5.69 ± 0.22 mg/dL.

From the results observed, it is stipulated that the mean salivary calcium and phosphate levels after chewing the gum containing calcium phosphate were numerically greater at all points of time when compared with levels before chewing the gum, with a mean increase in calcium levels at baseline, first week, and second week to be 0.80 ± 0.63, 0.974 ± 0.65, and 2.16 ± 1.79 mg/dL, respectively. This shows that chewing gum containing calcium phosphate is effective in increasing salivary calcium and phosphate levels in vivo as highly statistically significant increase in salivary calcium was achieved at all the three time intervals (P = 0.000). Similar results observed with phosphate levels were also numerically greater after chewing the gum at all points of time when compared with levels before chewing the gum, with a mean increase in phosphate levels at baseline, first week and second week observed to be 0.12 ± 0.23, 0.57 ± 0.37 and 0.96 ± 0.29 mg/dL respectively. This result shows highly statistically significant increase in salivary phosphate levels achieved at all the three time intervals (P = 0.000).

When we compared the baseline calcium and phosphate levels, the test chewing gum resulted in a significant increase in calcium and phosphate levels of 800 ± 0.63 and 0.12 ± 0.23 mg/dL (P = 0.000) [Table 1]. After application of ANOVA, there was no statistically significant difference among pre-calcium at baseline, first week, and second week (P = 0.076) [Table 2]. When we analyzed post-calcium and phosphate levels among different time intervals, a highly statistically significant difference was

| Variable                                      | Mean  | SD    | Paired differences | t     | Sig.  |
|-----------------------------------------------|-------|-------|--------------------|-------|-------|
| Mean difference                               |       |       | 95% CI of the difference |       |       |
| Lower                                        |       |       | Upper              |       |       |
| CAL levels - baseline score after chewing     | 10.48 | 0.97  | 0.800±0.63         | 0.636 | 0.964 | 9.77  | 0.000 |
| CAL levels - baseline score before chewing    | 9.68  | 1.13  |                    |       |       |       |       |
| P-levels - baseline score after chewing       | 4.80  | 0.11  | 0.125±0.23         | 0.064 | 0.185 | 4.14  | 0.000 |
| P-levels - baseline score before chewing      | 4.67  | 0.25  |                    |       |       |       |       |

SD: Standard deviation; CI: Confidence interval; CAL: Calcium

| Dependent variable | Comparison variable | Mean difference | Sig.  | 95% Confidence interval |
|--------------------|---------------------|----------------|-------|------------------------|
|                    |                     |                |       | Lower bound             | Upper bound |
| Baseline score - pre | First week - pre | −0.12325       | 0.846 | −0.6511               | 0.4046       |
|                     | Second week - pre  | 0.36752        | 0.229 | −0.1604               | 0.8954       |
| First week - pre   | Baseline score - pre | 0.12325       | 0.846 | −0.4046              | 0.6511       |
|                     | Second week - pre  | 0.49077        | 0.074 | −0.0371              | 1.0186       |
| Second week - pre  | Baseline score - pre | −0.36752     | 0.229 | −0.8954              | 0.1604       |
|                     | First week - pre   | −0.49077       | 0.074 | −1.0186             | 0.0371       |
observed \((P = 0.000)\). It was also observed that statistically significant increase in mean salivary calcium levels was found to be between baseline and second week, that is, 0.992 mg/dL \((p = 0.000)\) and between first and second weeks, that is, 0.695 mg/dL \((P = 0.001)\) [Table 3]. Similarly, when ANOVA was applied, it showed that there was no statistically significant difference among pre-phosphate levels at baseline, first week and second week \((P = 0.117)\) [Table 4], while we analyzed post-phosphate levels among different time intervals a highly statistically significant difference was observed \((P = 0.000)\). However the levels of salivary phosphate increased significantly at all the three points of time \((P = 0.000)\) [Table 5].

**Discussion**

Dental caries progressively lead to demineralization of tooth enamel that occurs when microorganisms metabolize dietary sugars into acids that release calcium and phosphate ions from hydroxyapatite. Dental caries is a highly prevalent disease and it is a major public health problem. For maximum remineralization to occur, calcium, phosphorus, and fluoride must all be available at sufficient levels in the oral environment.\(^9\)

Nowadays, three strategies are used for managing dental caries, that is, prevention, control and treatment, and it is based on appropriate methods of disease detection from the earliest stage as the lesion starts forming.\(^9\)

Recent concept uses the CPP-ACP molecule (Recaldent) which is a protein found in milk called casein which acts as a demineralizing agent and is remarkably capable of stabilizing calcium and phosphate. It is one of the promising noninvasive procedures for management of early carious lesions.

CPP-ACP possesses anticariogenicity properties as they act as a calcium phosphate reservoir, buffering the activities of free calcium and phosphate ions in plaque fluid and they maintain a state of supersaturation with respect to enamel minerals, and therefore they enhance remineralization and suppress demineralization.\(^4\)

Rose and Grychtol et al. demonstrated that CPP-ACP and calcium competed for the same binding sites on

| Table 3: Comparison of post-chewing Cal levels at baseline, 1st week and 2nd week scores using ANOVA |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Dependent variable | Comparison variable | Mean difference | Sig. | 95% Confidence interval |
| Baseline score - post | First week - post | −0.29713 | 0.268 | −0.7485 | 0.1542 |
| | Second week - post | −0.99282* | 0.000* | −1.4442 | −0.5415 |
| First week - post | Baseline score - post | −0.29713 | 0.268 | −0.1542 | −0.7485 |
| | Second week - post | −0.69568* | 0.001* | −1.1470 | −0.2443 |
| Second week - post | Baseline score - post | 0.99282* | 0.000* | 0.5415 | 1.4442 |
| | First week - post | 0.69568* | 0.001* | 0.2443 | 1.1470 |

ANOVA: analysis of variance. *P value ≤ 0.05 is considered to be statistically significant

| Table 4: Comparison of pre-chewing P levels at baseline, 1st week and 2nd week scores using ANOVA |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Dependent variable | Comparison variable | Mean difference | Sig. | 95% Confidence interval |
| Baseline score - pre | First week - pre | −0.08150 | 0.108 | −0.1764 | 0.0134 |
| | Second week - pre | −0.05717 | 0.331 | −0.1521 | 0.0378 |
| First week - pre | Baseline score - pre | 0.08150 | 0.108 | −0.0134 | 0.1764 |
| | Second week - pre | 0.02433 | 0.817 | −0.0706 | 0.1193 |
| Second week - pre | Baseline score - pre | 0.05717 | 0.331 | −0.0378 | 0.1521 |
| | First week - pre | −0.02433 | 0.817 | −0.1193 | 0.0706 |

ANOVA: analysis of variance

| Table 5: Comparison of post-chewing P levels at baseline, 1st week and 2nd week scores using ANOVA |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Dependent variable | Comparison variable | Mean difference | Sig. | 95% Confidence interval |
| Baseline score - post | First week - post | −0.52492* | 0.000* | −0.6373 | −0.4125 |
| | Second week - post | −0.89300* | 0.000* | −1.0054 | −0.7806 |
| First week - post | Baseline score - post | 0.52492* | 0.000* | 0.4125 | 0.6373 |
| | Second week - post | −0.36808* | 0.000* | −0.4805 | −0.2557 |
| Second week - post | Baseline score - post | 0.89300* | 0.000* | 0.7806 | 1.0054 |
| | First week - post | 0.36808* | 0.000* | 0.2557 | 0.4805 |

ANOVA: analysis of variance. *P value ≤ 0.05 is considered to be statistically significant
**Streptococcus mutans**.[9,10] CPP-ACP has been increasingly accepted as an adjunct to oral hygiene procedures. Chewing gum stimulates saliva and helps maintain buffer activity of saliva. It has been demonstrated evidently that CPP-ACP increases the level of calcium and phosphorus ion in plaque fivefold in humans in *in situ* studies and short-term mouthwash studies.[4]

However, Grychtol *et al.* found no significant differences between chewing gum containing calcium and calcium-free chewing gums with regard to change in mineral loss and lesion depth of enamel samples.[11]

This study stipulated that the mean salivary calcium and phosphate levels after chewing gum containing calcium phosphate were numerically greater at all time points when compared with levels before chewing the gum, with a mean increase in calcium levels at baseline, first week, and second week to be $0.80 \pm 0.63$, $0.974 \pm 0.65$, and $2.16 \pm 1.79$ mg/dL, respectively. This shows that chewing gum containing calcium phosphate is effective in increasing salivary calcium and phosphate levels *in vivo*.

Similar findings have been reported by various researchers in different parts of the world. Santhosh BP *et al.* reported 70.6% increase in salivary calcium concentration after chewing CPP-ACP Trident chewing gum. However, they found 28.3% decrease in phosphorus concentration after chewing CPP-ACP chewing gum.[12]

Ruchi Vashisht *et al.* applied GC Tooth Mousse 3 min thrice daily after meals for 14 days and they showed remineralization of the lesions and significant alterations seen in surface morphologies of enamel after 14 days of experiment.[13]

Similarly, Shen *et al.* found chewing sugar-free gum containing CPP-ACP produced 4-7 fold increase in salivary flow rate over a 2-min period. Preliminary studies show that no CPP-ACP could be detected in gum bolus after 10 min of chewing, hence in *in situ* studies they tested for 20 min asking to chew the gum and found enamel remineralization of 100%. On the basis of this study, the time chosen in this study is for 20 min, so that active ingredient of CPP-ACP gets properly dissolved into the saliva.[14]

Shen and Reynolds in their study revealed that CPP-ACP in a sugar-free chewing gum enhanced remineralization of enamel subsurface lesions by 100%–150%. They revealed a dose-related increase in enamel remineralization independent of chewing gum weight and type.[14] Similar results were obtained by D Gurunathan *et al.* and Carmen Ilena *et al.*.[2,15]

Reynolds EC had combined CPP-ACP nanocomplexes and fluoride in a toothpaste and found a greater increase in enamel remineralization.[16,17] Similar to our study, Catarina Ribeiro assessed remineralizing potential of CPP-ACP chewing gum. They found no active carious lesion at two levels, that is, at two and 24 h and suggested that chewing gum with CPP-ACP showed higher hardness recovery at both levels, that is, two and 24 h.[7]

In this study, two pellets of Trident chewing gum were given for 20 min for 15 days once daily and it was stipulated that the mean salivary calcium and phosphate levels after chewing the gum containing calcium phosphate were numerically greater at all time points when compared with levels before chewing the gum. But when pre-calcium and phosphorus levels were assessed at first and second weeks, no significant difference was seen in calcium and phosphorus levels; this may be due to removal of softened enamel (remineralized) which was produced by CPP-ACP trident chewing gum by tongue abrasion.[14] Similar reports were observed by E.C. Reynolds in 1997.[18]

F Cai incorporated CPP-ACP into the lozenges and found significantly increasing remineralization by 78% to 176%, respectively, after 14 days of consumption and they also found no significant relation between individual stimulated or unstimulated salivary flow rates. Dawes and Macpherson compared salivary stimulation between lozenges and sugar-free chewing gum and reported that consumption of sugar-free chewing gum produces significantly greater increase in salivary flow (3–4 mL/min) which was also reported by Shen *et al.*[14,19]

Reynolds E.C. in 1998 used 1.0% of CPP-ACP solution twice daily which resulted in a 144% increase in calcium level and a 160% increase in inorganic phosphate level which was similar to our study.[4,20]

**Limitations and implications**

This *in vivo* study investigated the effect of periodic CPP-ACP chewing gum as a remineralizing agent. But in every day situation, the enamel surfaces are bathed in saliva between the demineralization periods when dental plaque metabolizes sugar into acids. The pH stays low for some time but returns back to normal. In oral cavity, the lesion formed is covered by plaque and debris. This can make the situation altogether different from *in vivo* condition.

**Conclusion**

The introduction of CPP-ACP in preventive dentistry has provided a novel way of remineralizing dental tissues. The incorporation of CPP-ACP in chewing gum, lozenges, mouthwashes, dentifrices, and so on provides children with a new way to fight dental caries. Hence, the use of CPP-ACP as a preventive adjunct should be encouraged.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The
patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**
There are no conflicts of interest.

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