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Efficacy of casein derivate CPP-ACP[1]

Eficácia clínica do complexo de caseína CPP-ACP[1]

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ABSTRACT

Objective
A literature review of systematic reviews and in situ and in vivo randomized controlled trials was conducted in order to assess the role of casein phosphopeptide - amorphous calcium phosphate in providing caries-preventive effect superior to any intervention or placebo.

Methods
Initially, Pubmed database was searched for systematic reviews. Two systematic reviews were identified, which concluded that the quantity and quality of randomized controlled trials were insufficient to recommend the clinical use of the product. After this, Pubmed database was searched for in situ and in vivo randomized controlled trials that were not included in the reviews identified. In vivo and in situ studies yielded mixed results.

Results
In situ studies indicated greater efficacy of casein phosphopeptide - amorphous calcium phosphate in comparison with placebo. Whereas, in vivo studies demonstrated different findings

Conclusion
Casein phosphopeptide - amorphous calcium phosphate had a performance equivalent to or greater efficacy than placebo; fluoride was more effective than the casein complex; casein phosphopeptide - amorphous calcium phosphate associated with fluoride showed better or equivalent performance to that of placebo. In view of available evidence, it was not possible to generalize the indication of casein phosphopeptide - amorphous calcium phosphate for preventing dental caries. Further studies on the preventive effect and longer treatment applications are recommended.

Indexing terms: *Phosphopeptides. Sodium fluoride. Tooth remineralization.*

RESUMO

Objetivo
Foi conduzida revisão da literatura em busca de ensaios clínicos randomizados *in situ* e *in vivo* que avaliassem o papel do complexo de fosfopeptídeo de caseína - fosfato de cálcio amorfo na prevenção de lesões de cárie.

Métodos
Inicialmente, buscou-se identificar na base *Pubmed* revisões sistemáticas sobre o tema. Foram identificadas duas revisões sistemáticas, que concluíram que a quantidade e qualidade dos ensaios clínicos randomizados são insuficientes para recomendação do uso clínico do produto. Posteriormente, pesquisaram-se ensaios clínicos randomizados *in situ* e *in vivo* que não tivessem sido incluídos nas revisões identificadas.

Resultados
Os estudos *in vivo* e *in situ* tiveram resultados contraditórios. Os estudos *in situ* indicaram uma maior eficácia do complexo de fosfopeptídeo de caseína - fosfato de cálcio amorfo em relação ao placebo. Já os estudos *in vivo* demonstraram diferentes.

Conclusão
Complexo de fosfopeptídeo de caseína - fosfato de cálcio amorfo apresentou desempenho ora superior, ora equivalente ao placebo; o flúor demonstrou maior eficácia que o complexo de caseína; o complexo de fosfopeptídeo de caseína - fosfato de cálcio amorfo associado ao flúor se mostrou ora melhor, ora equivalente ao placebo. Diante da evidência científica disponível, não se pode generalizar a indicação do complexo de fosfopeptídeo de caseína - fosfato de cálcio amorfo para prevenção de lesões cariosas.

Termos de indexação: Fosfopeptídeo. Fluoreto de sódio. Remineralização dentária.

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INTRODUCTION

Caries: processes of dental remineralization and demineralization

According to the World Health Organization (WHO), in 2003, caries diseases was the major public health problems in the majority of industrialized countries. It affects 60 to 90% of children of school-going age, and the large majority of adults[1].

Caries is known to be a multifactorial disease. The development of caries lesions depends on interaction between the tooth structure, cariogenic microorganisms (particularly *Streptococcus mutans*,) and presence of fermentable carbohydrates. Therefore, preventive and therapeutic approach must consider the set of these factors.

Cariogenic microorganisms colonize the tooth surface and form dental biofilm. In physiologic conditions, the oral fluids (saliva and biofilm) present a higher concentration of calcium (Ca) and phosphate (P) in the medium outside of the tooth (supersaturation) than in hydroxyapatite (HA), which is the primary constituent of the enamel structure (crystallized form of calcium phosphate) These ions are continually deposited on the tooth surfaces and in areas in which demineralization processes occur. This process is known as the "natural defense phenomenon" promoted by saliva to promote the mineral preservation of tooth enamel[2].

The loss (demineralization) and gain (remineralization) of minerals on the enamel surface is a dynamic physical-chemical process, which occurs when the bacteria present in dental biofilm are exposed to a diet composed of fermentable carbohydrates, particularly sucrose. Whenever sugar penetrates into cariogenic biofilm, it is converted into acid and the biofilm fluid becomes saturated in comparison with the mineral component of enamel. In this situation, pH has a low critical value, at which demineralization of enamel occurs. However, after a certain period, the physiological value of pH is restored when sugar consumption ceases, and the conditions of supersaturation are re-established. At this time,, reposition of a certain quantity of lost mineral occurs, in a process denominated dental remineralization. This reposition of lost mineral occurs by means of Ca and P ions present in biofilm fluid and saliva, right after dental biofilm removal by brushing. The quantity of Ca and P replaced is lower than the amount lost, so that small mineral losses occur[2].

If the factors responsible for disease (biofilm and frequent exposure to sugar) are not controlled, with the passage of time, mineral loss cannot be impeded. Therefore, events of mineral dissolution will repeatedly occur, and may lead to a degree of demineralization greater than that of the remineralizing capacity of the oral fluids. Therefore, an imbalance in these processes of demineralization and remineralization may result in small mineral losses, only observed by electronic or optical microscopy; mineral losses observed clinically (white spots), up to the formation of cavities on the tooth surface, in which it will be necessary to perform invasive interventions. Moreover, it is important to point out that caries disease progresses in a different manner among individuals, as it is a disease in which individual susceptibility is also an important factor for its progression.

Fluoride

At present, it is known that the action of fluoride (F) in interfering in the profess of caries lesion formation is not systemic, but local. For this purpose, fluoride must be present in the biofilm and saliva at the time in which the biofilm is exposed to sugar, or after its removal during tooth brushing[2].

It is noted that fluoride, even in low concentrations, interferes in the process of caries development. Hydroxyapatite (HA) dissolves at a pH of around 5.5, while fluorapatite (FA: crystallized form of F, Ca and P) dissolve at a pH close to 4.5. When the oral pH remains between 4.5 and 5.5, the process of HA demineralization occurs, in which there is release of Ca, P and hydroxyls in the oral environment. If there is fluoride present, these ions react with it and fluorapatite is formed which, saturated at this pH, is deposited on the tooth surface. This compensates the mineral loss occurring at pH between 4.5 and 5.5[3]. However, this mineral reposition occurring by means of fluorapatite formation is not considered remineralization, in fact. but rather as an inhibition of demineralization, because the mineral component deposited differs from the one lost. Furthermore, fluorapatite is deposited on the tooth surface, while the Ha is dissolved in the subsuperficial region of the tooth[2].

As previously stated, caries is essentially a disease related to tooth demineralization. A considerable body of literature has established the use of fluoride as being an important agent in dental remineralization. The interaction between the ions of Ca and fluoride, which form fluorapatite, is greater between the ions of Ca and OH, which forms HA. This gives fluorapatite greater stability and lower solubility. Therefore, fluoride is the main component of dentifrices and mouth washes[4].

The indirect effect of fluoride on the reduction of dental demineralization, when pH falls, is complemented by the natural effect of fluoride on dental remineralization,

when the pH rises, promoting the reposition of Ca and P ions present in biofilm fluid. If the demineralized surface is submitted to tooth brushing, the saliva is capable of promoting remineralization. However, if there is presence of fluoride, this process is potentiated[2].

Dentifrices and fluoridated oral solutions have been demonstrated to diminish the activity of caries in controlled randomized clinical trials. The efficacy of these products arises from their ability to incorporate fluoride ions in the plaque and tooth enamel[5]. However, the toxicological potential of fluoridated compounds must be pointed out. There is risk of acute intoxication occurring when a large quantity of fluoride is ingested. There may also be chronic intoxication when there is consumption of a concentration of fluoride in excess of the adequate amount for a longer period of time.

CPP-ACP

In view of the cariogenic challenges to which dental structures are usually submitted, one notes that there has been an endeavor to develop methods for the diagnosis of caries lesions in the initial stages, in order to prevent their progression, and consequently, the need for restorative intervention. In addition, different preventive therapies have been studied, with the potential to increase the degree of remineralization, reduce the process of demineralization and therefore, promote reduction in the incidence of active caries lesions[6].

Recently, many studies have centered their evaluations on the concentration of Ca and P present in the teeth. These ions are the main dental components and are intimately related to the process of demineralization. Therefore, many efforts have been concentrated on analysis of the deposition of these minerals on the dental structure, and on agents that may promote the availability of these ions in the oral environment[6].

There have been demonstrations that the complex of casein phosphopetide (CPP) - amorphous calcium phosphate (ACP) has an anticariogenic activity in laboratory experiments in animals and humans. The potential of CPP-ACP to inhibit demineralization and to stimulate remineralization is based on the ability of CPP to stabilize ACP, and later formation of CPP-ACP. This complex acts as a reservoir of Ca and P that bonds to the plaque and tooth surface. In the face of an acid medium CPP-ACP releases Ca and P ions, so that mineral supersaturation is maintained in the environment outside of the tooth, and consequently, reduction in the demineralization process and stimulation of remineralization is achieved[7-8].

Furthermore, the nanocomplex of CPP-ACP has been shown to have a synergic effect on fluoridated compounds. This synergic effect must be attributed to the formation of nano-agglomerates of Ca, F and P ions (CPP-ACP/F), which results in greater incorporation of fluoride ions into plaque, together with the increase in the concentration of bioavailable Ca and P ions[5].

The intervention based on CPP-ACP most commonly used in *in vivo* studies occurs by means of chewing gums. Other CPP-ACP vehicles include mouth washes and topical cream, denominated *Tooth Mousse*™ (Europe and Australia) or *MI Paste*™ (United States of America and Japan).

METHODS

The review of the literature was conducted with the aim of verifying the clinical efficacy of CPP-ACP in inhibiting the formation of caries lesions.

Initially, a search was conducted in the electronic databases of *Pubmed, The Cochrane Library and Centre for Reviews and Dissemination (CRD*) for systematic reviews (SR), with or without meta-analyses, which evaluated the efficacy of CPP-ACP (Table 1).

Table 1. Description of search strategy for systematic reviews

Bases	Terms	Results	Studies selected
Medline (via Pubmed)	"casein phosphopeptide-amorphous calcium phosphate nanocomplex" [substance] OR "CPP ACP"[All Fields] AND ((meta analysis[ptyp] OR meta-analysis[tiab] OR meta-analysis[mh] OR (systematic[tiab] AND review[tiab]) NOT ((case[ti] AND report[ti]) OR editorial[ptyp] OR comment[ptyp] OR letter[ptyp] OR newspaper article [ptyp]))))	5	2
Cochrane Library (via Bireme)	*CPP ACP*	1 systematic review protocol	-
Centre for Reviews and Dissemination	*CPP ACP*	0	0

Afterwards a search was conducted in *Pubmed* for randomized clinical trials (RCT) (*in vivo* or *in situ*) which had not been included in the systematic reviews identified and which evaluated the efficacy of CPP-ACP, in any application vehicle (Table 2).

Table 2. Description of search strategy for randomized clinical trials.

Bases	Terms	Results	Studies selected
Medline (via Pubmed)	"casein phosphopeptide-amorphous calcium phosphate nanocomplex"[Substance] OR "CPP ACP"[All Fields] AND (randomized controlled trial[Publication Type] OR (randomized[Title/Abstract] AND controlled[Title/Abstract] AND trial[Title/Abstract]))	39	13

In addition, as the articles published in the year 2012 were still in the process of indexation by *Pubmed*, a more sensitive search was conducted by means of using the terms "*casein phosphopeptide-amorphous calcium phosphate nanocomplex*"[Substance] OR "*CPP ACP*"[All Fields], for the year 2012. Nine publications were found, and 2 *in situ*[20] study was selected, in accordance with the above-mentioned inclusion criteria.

RESULTS

Systematic reviews

Initially, 2 systematic reviews were identified. The first of these, conducted by Azarpazhooh et al.[7], reunited randomized and quasi randomized clinical studies, published up to October 2007, which tested the efficacy of casein derivatives (among them CPP-ACP) in the diverse forms of dental application.

In this review, 12 studies were selected, which tested the efficacy of casein derivatives in dental clinical practice. These studies were selected by means of the inclusion criterion, and measurement of the force and quality of the studies, in accordance with the System of Classification of Evidence, developed by the *Canadian Task Force* for preventive treatments. The outcomes of interest were as follows: prevention of caries (10 studies), treatment of dentinal hypersensitivity (1 study) and treatment of xerostomia (1 study).

Of the ten studies that had prevention of caries as the outcome, eight were *in situ* and two were *in vivo* studies. Among the latter studies mentioned, one of them were related to the regression of white spot lesion[9]. The other *in vivo* study evaluated the regression or progression or root caries lesions with the use of a mouth wash solution based on casein derivatives associated with calcium phosphate (CD-CP), in comparison with the use of a fluoride-based solution, in individuals with salivary gland dysfunctions[10] (Table 3).

Table 3. Studies *in vivo* and *in situ* found in the review by Azarpazhooh et al.[7].

Author/Year	Population	Intervention	Study period*	Outcomes	Type of study
Andersson et al.[9]	26 adolescents (60 teeth, 152 visible white spot lesions)	*Test Group* Brushing twice a day with CPP-ACP-based cream for 3 months, followed by use of fluoridated dentifrice for a further 3 months. *Control Group* Fluoridated mouth wash once a day in addition to the daily use of fluoridated dentifrice for six months.	12 months	Visual inspection and evaluation by means of fluorescent laser of white spot lesions in time intervals of 1, 3, 6 and 12 months after beginning of intervention.	RCS *in vivo* mono-blind
Hay & Thomson[10]	124 patients with salivary gland dysfunction	*Test Group* CD-CP-based mouth wash (casein derivative associated with calcium phosphate) 3 times a day. *Control Group* Fluoridated mouth wash 3 times a day.	12 months	Reduction of root caries lesion (Bite-wing radiograph) after 12 months	RCS *in vivo* double-blind (blinding not clear)
Cai et al.[3]	10 adults	Chewing gum without sugar + 20 mg citric acid + 18.8 mg of CPP-ACP Chewing gum + 20 mg of citric acid Chewing gum without addition of citric acid and CPP-ACP	14 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Schirrmeister et al.[11]	15 adults	CPP-ACP-based chewing gum	21 days	Reduction in depth of lesion	RCS *In situ* mono-blind
Walker et al.[12]	10 adults	*Milk* With addition of 2g CPP/ACP/l With addition of 5g CPP/ACP/l Without addition of CPP/ACP/l	15 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Itthagarun et al.[13]	12 adults	Sugar-free chewing gum with: 30 mg of urea 30 mg of urea + 25mg of calcium phosphate 30 mg of urea + 47 mg of CPP-ACP	21 days	Reduction in lesion depth (remineralization of lesions *in situ*)	RCS *In situ* double-blind
Iijima et al.[14]	10 adults	Chewing gum: With addition of CPP-ACP (18.8MG) Without addition of CPP-ACP	14 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind

cont.

cont.

Cai et al.[15]	30 adults	*Study 1* Mouth wash 2% CPP-ACP 6% CPP-ACP Calcium + phosphate Placebo *Study 2* CPP-ACP-based chewing gum or other calcium-based compound	*Study 1* 5 days *Study 2* 14 days	*Mouth wash* Level of phosphate and calcium present in dental plaque *Chewing gum:* Degree of subsuperficial remineralization	RCS *In situ* double-blind
Cai et al.[16]	10 adults	*Chewing gum:* With addition of 18.8mg CPP-ACP With addition of 56.4mg CPP-ACP Without addition of CPP-ACP	14 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Shen et al.[17]	30 adults	*Chewing gum:* Sorbitol ('pellet gum') + 4 different doses of CPP-ACP Sorbitol ('slab gum') + 4 different doses of CPP-ACP Xylitol + 4 different doses of CPP-ACP 4 different doses: 0; 0,19; 18,8 and 56,4 mg CPP-ACP	14 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind

Note: Period of each of the interventions tested.

With regard to *in situ* studies, the patients used an acrylic device that covered the palate from the first premolar to the last tooth in the dental arch, into which fragments of human teeth were fitted, which had demineralized subsuperficial lesions, in 7 of the 8 *in situ* studies identified. Only in the study of Schirrmeister et al.[11] was a fragment of bovine tooth used in the acrylic devices fitted to the mandible.

With regard to the conclusions of these studies, only the study of Schirrmeister et al.[11] did not observe a higher rate of demineralization in the subsuperficial lesions evaluated. All the other seven *in situ* studies observed a significant increase in the degree of remineralization of the lesions treated with casein compounds.

Only one *in vivo*[9] study was identified, which tested the efficacy of CPP-ACP compounds in the process of caries lesions, in comparison with fluoridated compounds. Visual inspection and evaluation by means of fluorescent laser were performed in time intervals of 1, 3, 6 and 12 months after beginning of intervention. There was only statistical significance that benefitted CPP-ACP (63%) in the reduction of white spots in comparison with fluoridated compounds (25%) in the evaluation that used visual inspection performed 12 months after the beginning of treatment. Whereas, the method that used fluorescent laser was not capable of identifying any difference between the treatments[9].

The review of Azarpazhooh et al.[7] concluded that the quality and quantity of articles reviewed were insufficient to generate conclusive evidence of the long term effectiveness of casein derivative compounds, especially CPP-ACP.

The other review found was associated with a meta-analysis. In this study, Yengopal & Mickenautsch[8] included clinical studies (*in situ and in vivo*) and systematic reviews, published up to August 2008, which evaluated the efficacy of CPP-ACP-based compounds. In order to be selected, the studies needed to make information available with respect to sample size, loss of follow-up of patients and about the follow-up period of the study. Eleven studies and one systematic review[6] were selected. All of the 11 studies selected presented Score A, on a scale of evaluation, with regard to randomization, confidentiality in allocation and blinding. Only two studies presented Score B for the parameter confidentiality in allocation. Of these 11 studies, 5 participated in the meta-analysis, as they were the only one with characteristics that allowed statistical analysis of their results in conjunction; that is, they were clinically and methodologically homogeneous, and presented similar outcomes.

All of the five[3,14,16-18]studies included in the meta-analysis were of the *in situ* type, with a short duration of follow-up (7 to 21 days), and the intervention used was chewing gum containing CPP-ACP. The result of this analysis showed higher rates of tooth remineralization in favor of the use of CPP-ACP-based chewing gums, in comparison with the use of chewing gum without the addition of CPP-ACP, or in comparison with no intervention. Among the other 6 studies not included in the meta-analysis, 4[12-13,15] were *in situ* studies, and all of them showed a higher rate of remineralization with the use of CPP-ACP. However, it is worth emphasizing that one of these studies[4] compared CPP-ACP added to fluoride in comparison with CPP-ACP alone and with

fluoride alone, As a result, CPP-ACP associated with fluoride was observed to be the intervention that showed the best performance, while the intervention based on CPP-ACP only had a similar performance to that of fluoride alone.

The other two studies not included in the meta-analysis were *in vivo.* One of them was the study that was included in the systematic review of Azarpazhooh et al.[7], which was that of Anderson et al.,[9]. The other study refers to that of Morgan et al.[19], in which 2,720 children used chewing gum with or without the addition of CPP-ACP. The follow-up period of the study was 24 months, and regression of caries lesions was observed by means of bite-wing radiographs that allowed visualization of the interproximal dental regions.

It was observed that of the nine*in situ* studies found in the review of Yengopal & Mickenautsch[8], seven studies[3,12,17] coincided with those already selected by the review of Azarpazhooh et al.[7]; the other two studies found only in the more recent review were published after the period of search conducted by Azarpazhooh et al.[7].

As follows below, there is information about these two new *in situ* studies and the new *in vivo* study found by Yengopal & Mickenautsch[8] (Table 4).

Table 4. Studies *in vivo* and *in situ* found in the review by Yengopal & Mickenautsch[8].

Author/Year	Population	Intervention	Study period*	Outcomes	Type of study
Reynolds et al.[5]	14	*Study 1* Mouth wash based on CPP-ACP(2%) + 450 ppm Fluoride Mouth wash based on 450 ppm Fluoride Placebo Mouth wash *Study 2* Dentifrice 1000 ppm F, as NaF 2800 ppm F, as NaF 2%CPP-ACP 2%CPP-ACP + 1100ppm F as NaF	*Study 1* 4 days *Study 2*, 14 days	*Study 1* Level of fluoride present in dental plaque *Study 2* Degree of subsuperficial remineralization	RCS *in situ* with *in vivo* stage double-blind
Manton et al.[18]	10	3 types of chewing gum Two chewing gums based on Sorbitol/ Xylitol without addition of CPP-ACP CPP-ACP-based chewing gum	14 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Morgan et al.[19]	2720	Sorbitol-based chewing gum without addition of CPP-ACP, 3 times a day (894 patients completed the treatment); CPP-ACP-based chewing gum, 3 times a day (926 patients completed the treatment);	24 months	Progression or regression of caries lesions observed by means of interproximal radiographs	RCS *In vivo* double-blind

Note: Period of each of the interventions tested.

Yengopal & Mickenautsch[8] observed that in spite of the meta-analysis having been conducted only for *in situ* studies that presented a short period of exposure to this complex, the promising results of the *in vivo* studies, one of them with a large sample size[19] and both[9,19] with a longer time of exposure to the CPP-ACP complex (12 to 24 months), suggested the remineralizing effect of the CPP-ACP complex, in studies with long follow-up periods. It is suggested that further randomized *in vivo* studies, with adequate methodology and longer follow-up periods should be conducted.

Studies *in vitro* and *in vivo*

In addition to the systematic reviews cited, as mentioned in the item Methodology, a new search was conducted in the *Pubmed* database in May 2012, in order to identify *in situ* and *in vivo* randomized clinical trials that evaluated the remineralizing potential of CPP-ACP, published after the search conducted by the last systematic review published about the subject (after the month of August 2008). Six *in situ* and 7 *in vivo* studies were identified (Table 5)

With regard to the conclusion of the 6 *in situ* studies, all of them showed a higher rate of remineralization with the use of CPP-ACP alone or associated with F. However, it is important to point tout that four of these studies compared CPP-ACP with a placebo, while two studies[21,23] compared interventions of CPP-ACP associated with fluoride, in comparison with CPP-ACP alone. In only one of them[21] was the comparison with fluoride alone, the results of which demonstrated that the intervention based on CPP-ACP associated with fluoride was the one that obtained the best results, followed by CPP-ACP alone, which in turn showed a better performance than that of fluoride alone.

Table 5. Studies *in vivo* and *in situ* found in search conducted in Pubmed.

Author/Year	Population	Intervention	Study period*	Outcomes	Type of study
Cochrane et al.[20]	9	Chewing gum with addition of CPP-ACP Chewing gum without addition of CPP-ACP Control Group without use of chewing gum	14 days	Degree of remineralization	RCS *In situ* double-blind
Shen et al.[21]	5	*Cream/mousse based on* CPP-ACP Fluoride 900 ppm associated with CPP-ACP Fluoride 1000 ppm Fluoride 5000 ppm Climpro 950 ppm Placebo	10 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Walker et al.[22]	*Study 1* 10 participants *Study 2* 14 participants	In both studies, CPP-ACP-based "candies" were used: *Study 1* Control (65% sucrose + 33% glucose); Sugar + 0.5% (w/w) CPP-ACP; Sugar + 1.0% (w/w) CPP-ACP; -ACP Control free of sugar *Study 2* Control (65% sucrose + 33% glucose syrup); Sugar free + 0.5% (w/w) CPP Sugar + 1.0% (w/w) CPP-ACP; Control free of sugar	10 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Srinivasan et al.[23]	5	Cream/mousse based on CPP-ACP Cream/mousse based on CPP-ACP and fluoride 900 ppm Cream/mousse placebo *Treatment occurred 1 time per day for 3 minutes	2 days	Degree of subsurface remineralization in teeth that suffered erosion	RCS *In situ* mono-blind
Walker et al.[24]	10	100 mL cows milk based on CPP-ACP (0.2%) 100 mL cows milk based on CPP-ACP (0.3%) 100 mL cows milk without addition of CPP-ACP	15 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Cai et al.[25]	10	Chewing gum with or without addition of CPP-ACP	14 days	Degree of subsuperficial remineralization	RCS *In situ* double-blind
Robertson et al.[26]	60	CPP-ACP-based cream with addition of fluoride Cream placebo In both groups the product was applied 1 time per day, after night time brushing.	3 months	Prevention and reduction of white spots.	RCS *In vivo* double-blind
Altenburge et al.[27]	32	Tooth brushing with fluoridated dentifrice and use of CPP-ACP-based cream, for 3 minutes, 1 time per day. Tooth brushing with fluoridated dentifrice	3 weeks	Evaluation of initial lesion of fissures in molars and premolars before and after treatment	RCS *in vivo* mono-blind
Beerens et al.[28]	54	CPP-ACP-based cream, associated with fluoride, applied before going to sleep. Placebo creamed based on calcium, applied before going to sleep	3 months	Degree of remineralization of white spot lesions and composition of bacterial plaque	RCS *In vivo* double-blind
Uysal et al.[29]	14	The brackets were bonded to premolars with 2 types of cement: Aegis Ortho® (composition based on ACP) Concise® resin cement	30 days	Reduction of dental demineralization around orthodontic brackets	RCS *in vivo* mono-blind
Uysal et al.[30]	21 patients 60 teeth	Cream based on CPP-ACP Fluoride in gel form No product was applied in the control group	60 days (*in vivo* phase) 14 days (*in vitro* phase)	Reduction of dental demineralization around orthodontic brackets	RCS *in vivo and in vitro* mono-blind
Bröchner et al.[31]	60 patients	Tooth brushing with fluoridated dentifrice in the morning, and application of CPP-ACP-based cream at night. Tooth brushing with fluoridated dentifrice in the morning, and at night.	4 weeks	Degrees of remineralization of white spot lesions	RCS *in vivo* mono-blind
Bailey et al.[32]	45 patients	Cream based on CPP-ACP Cream placebo *Application was made twice a day, after tooth brushing with fluoridated dentifrice	12 weeks	Regression of white spot lesions	RCS *In vivo* double-blind

Note: Period of each of the interventions tested.

Among the 7 *in vivo* studies, 2 of them[26,28] evaluated the use of CPP-ACP associated with fluoride, which was shown to be more effective than the placebo in only one of the studies[26]. In the study of Beerens et al.[28] there was no significant difference between the interventions.

Of the other 5 *in vivo articles*[27,29-32], in only one of them[30] was an intervention used in comparison with one based on F, which was shown to be as effective as CPP- ACP. Among the four remaining studies, in which the placebo was used for comparison, in three of these[27,29,32] CPP-ACP demonstrated a higher remineralization potential. However, in one of these studies[27] the greater efficacy of CPP-ACP was observed only when some evaluations were made by means of fluorescent laser. Nevertheless, there was no significant difference between the treatments when visual evaluation was made. In the remaining study[31], CPP-ACP showed the same performance as the placebo.

DISCUSSION

The process of enamel remineralization has been studied for over 100 years. It has been suggested that non invasive treatment of initial caries lesions occurring by means of processes that stimulate remineralization, has been one of the major advances in control of the disease.

Therefore, the appearance of products that have the potential to promote dental remineralization are welcome, however, they need to be evaluated with caution, by means of analysis of the best scientific evidence available.

CPP-ACP has been extensively studied recently. In order to better evaluate its remineralizing potential, this literature review was conducted with the goal of seeking clinical results of the efficacy of this complex in the prevention of caries lesion formation.

Both meta-analyses identified, suggested that further *in vivo* studies with long follow-up periods should be conducted in order to reduce the uncertainty about the clinical efficacy of CPP-ACP, particularly in comparison with fluoridated compounds.

As regards the *in situ* studies found, CPP-ACC demonstrated greater efficacy than the placebo in the majority of publications. The greater efficacy of CP-ACP associated with fluoride in comparison with CPP-ACP alone was also demonstrated. In only one study was the superiority of the casein complex verified in comparison with fluoride, considered the standard therapy in the process of dental remineralization.

Whereas, with reference to the *in vivo* studies, findings with a higher level of scientific evidence, the results were contradictory: CPP-ACP associated with fluoride was at times shown to be more effective, and at times was shown to be similar to the placebo. CPP-ACP alone at times demonstrated greater efficacy, at time demonstrated similarity to placebo, and when compared with F, obtained an equivalent performance.

In view of the data presented, up to the moment, it has not been conclusively demonstrated that CPP-ACP (alone or in association with fluoride) has advantages in comparison with fluoride in the promotion of dental remineralization. Indeed, there are also data that demonstrate clinical similarity of the casein complex in comparison with the placebo.

Moreover, the posology of CPP-ACP was noted to be variable. The best form and frequency of application were not established. This fact strongly influences the comparison of the results found in the studies, which used different polsologies of the product.

In spite of the majority of studies comparing CPP-ACP with a placebo, it is very important for the substance used for comparison to be standard therapy, in this case, fluoridated compounds. Because, in spite of noting the appearance of new products with remineralizing potential, it is not always possible to prove clinical advantages of the new in comparison with existent therapies.

CONCLUSION

A cautious approach is needed when making a general recommendation about the use of CPP-ACP in the prevention of caries, considering that there is no uniformity in the results of studies with a higher level of evidence, with respect to the remineralizing role of the casein complex in clinical practice.

It is suggested that the results of further *in vivo* studies, with long follow-up be awaited, in order to be able to generalize the benefit demonstrated by CPP-ACP in some patients.

Collaborators

RF PEREIRA conducted the bibliographic review for preparing the introductory part, under the supervision of the author SC LEAL. RF PEREIRA and SC LEAL were involved in developing the search strategies, selection of articles and interpretation of their results. Afterwards, discussions were held about the findings, and the article presented was written.

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