Clinical efficacy of casein derivatives
A systematic review of the literature

Amir Azarpazhooh, DDS, MSc; Hardy Limeback, BSc, PhD, DDS

ABSTRACT

JADA CE CONTINUING EDUCATION ARTICLE 3

Background. The objective of this article was to review systematically the clinical trials of casein derivatives (specifically casein phosphopeptide–amorphous calcium phosphate [CPP-ACP] complex) used in dentistry.

Types of Studies Reviewed. The authors included clinical studies that examined the efficacy of casein derivatives in dentistry. They excluded in vitro studies, case series, case reports, letters to editors (not containing primary data), editorials, review articles and commentaries, but read them to identify any potential studies.

Results. The authors searched 98 articles for relevance, determined according to title, abstract and full text, resulting in a yield of 12 original studies. Nine were clinical trials that focused on caries prevention, seven of which showed that CPP-ACP (as found in sugar-free pellet or slab chewing gum, lozenges, milk or mouthrinse) was effective in preventing dental caries by remineralizing subsurface carious lesions in situ in a dose-response fashion. One was a clinical trial with conflicting results regarding the effect of CPP-ACP on the regression of white-spot lesions; one was a survey of the relief of dry-mouth symptoms; and one was an uncontrolled clinical study that showed the lack of effectiveness and lack of short-term therapeutic effect in treating dentin hypersensitivity.

Clinical Implications. The quantity and quality of clinical trial evidence are insufficient to make conclusions regarding the long-term effectiveness of casein derivatives, specifically CPP-ACP, in preventing caries in vivo and treating dentin hypersensitivity or dry mouth.

Key Words. Anticariogenicity; casein derivative; casein phosphopeptide–amorphous calcium phosphate; remineralization; demineralization; systematic review.
JADA 2008;139(7):915-924.

Casein is the predominant phosphoprotein in bovine milk and accounts for almost 80 percent of its total protein, primarily as calcium phosphate stabilized micellular complexes.[1] Several laboratory and animal experiments[2-9] have investigated the low cariogenic potential and the possible cariostatic activity of dairy products (milk, casein, caseinates and cheeses). Casein phosphopeptides (CPP) contain the cluster sequence of -Ser (P)-Ser (P)-Ser (P)-Glu-Glu from casein.[10,11] Through these multiple phosphoseryl residues, CPP can remarkably stabilize calcium phosphate (which usually is highly insoluble) in a state-forming CPP-amorphous calcium phosphate (ACP) complex.[12,13] This complex is a nanocluster of ACP with four multiphosphorylated peptides that prevent its growth to the critical size required for nucleation, phase transformation and precipitation.[13,14]

On the basis of the generally accepted molecular formula for ACP [$Ca_3(PO_4)_2 \cdot nH_2O$], ACP also may be considered a tricalcium phosphate. There is no conclusive evidence that ACP is an integral mineral component in hard tissues. It likely plays a special role as a precursor to bioapatite and as a transient phase in biomineralization. In solutions, ACP is converted readily to stable crystalline phases such as

Dr. Azarpazhooh is a PhD/specialty candidate in endodontics and a researcher, Community Dental Health Services Research Unit, Faculty of Dentistry, University of Toronto, Room 521A, 124 Edward St., Toronto, Ontario, M5G 1G6, Canada, e-mail "amir.azarpazhooh@dentistry.utoronto.ca". Address reprint requests to Dr. Azarpazhooh.
Dr. Limeback is an associate professor and head, Preventive Dentistry, Faculty of Dentistry, University of Toronto.

octacalcium phosphate or apatitic products.[15]

The CPP-ACP complex was patented by the University of Melbourne, Australia, and the Victorian Dairy Industry Authority, Abbotsford, Australia. Bonlac Foods Limited (an Australian company owned by 2,300 dairy farmers in Victoria and Tasmania) has exclusive manufacturing and marketing rights for CPP-ACP and is the owner of the trademark (Recaldent). In early 1999, the U.S. Food and Drug Administration (FDA) accepted Recaldent as "generally recognized as safe" for its intended use as a texturizer in chewing gum (Trident White, Cadbury Adams USA, Parsippany, N.J.) at up to 5 percent weight per weight.[16]

The FDA has approved products marketed in the United States (MI Paste and MI Paste Plus containing 900 parts per million fluoride, GC America, Alsip, Ill.) for use primarily as abrasive prophylaxis pastes and secondarily for the treatment of tooth sensitivity (after in-office bleaching procedures, ultrasonic scaling, hand scaling or root planing). However, its use for remineralizing dentin and enamel and preventing dental caries is an off-label application.[17] Outside the United States, the products are marketed as GC Tooth Mousse and Tooth Mousse Plus (GC Europe N.V., Leuven, Belgium).

Several laboratory and animal experiments have demonstrated the anticariogenic potential of casein phosphopeptide–amorphous calcium phosphate.

RATIONALE FOR USE OF CASEIN DERIVATIVES

Although several studies have investigated the use of casein[4,18-23] as an anticariogenic additive to food, toothpaste or drinking water, its use has not been implemented because of its adverse organoleptic properties and the large amount required for efficacy.[13] In contrast, CPP does not have these limitations. The potential for a specific anticariogenic activity is at least 10 times greater on a weight basis for CPP than it is for casein. Therefore, CPP can be used as a food or toothpaste anticariogenic additive, especially if it is consumed at the same time as the cariogenic challenge.[13]

Reynolds and colleagues[12,24] reported that CPP-ACP binds readily to the surface of the tooth, as well as to the bacteria in the plaque surrounding the tooth. In this way, CPP-ACP deposits a high concentration of ACP in close proximity to the tooth surface. The authors proposed that under acidic conditions, this localized CPP-ACP buffers the free calcium and phosphate ions, substantially increasing the level of calcium phosphate in plaque and, therefore, maintaining a state of supersaturation that inhibits enamel demineralization and enhances remineralization.

Several laboratory and animal experiments have demonstrated the anticariogenic potential of CPP-ACP. In a group of specific-pathogen-free rats inoculated with *Streptococcus sobrinus*, Reynolds and colleagues[25] applied CPP-ACP solution to the animals' molar teeth twice daily. They found that the caries activity of the enamel smooth surfaces was reduced significantly in a dose-response fashion (0.1 percent and 1.0 percent weight per volume CPP-ACP, respectively, produced a 14 percent and a 55 percent reduction in smooth-surface caries activity). The authors found a similar reduction in the caries activity of fissures (0.1 percent and 1.0 percent w/v CPP-ACP, respectively, produced a 15 percent and a 46 percent reduction in fissure caries activity).

In an in vitro study, Reynolds[26] demonstrated that CPP-stabilized calcium phosphate solutions maintained high concentration gradients of calcium and phosphate ions and ion pairs in subsurface carious lesions in the enamel of human third molars, resulting in high rates of enamel remineralization. The remineralizing capacity was greater for solutions with higher levels of CPP-stabilized free calcium and phosphate ions.

Rose[27] conducted a laboratory experiment in which he showed that CPP-ACP binds well to dental plaque, providing a large calcium reservoir that may inhibit demineralization and assist in subsequent remineralization. In a second experiment, Rose[28] also showed that in streptococcal model plaques, 0.1 percent CPP-ACP provides a

ABBREVIATION KEY. $CaCO_3$: Calcium carbonate. **$CaHPO_4/CaCO_3$:** Calcium hydrogen phosphate/calcium carbonate. **CD-CP:** Casein derivatives coupled with calcium phosphate. **CPP-ACP:** Casein phosphopeptide–amorphous calcium phosphate. **FDA:** Food and Drug Administration. **NaF:** Sodium fluoride. **RCT:** Randomized controlled trial. **WSL:** White-spot lesion.

large number of possible binding sites for calcium and reduces the free calcium diffusion coefficient by about 65 percent at pH 7 and 35 percent at pH 5. During a cariogenic episode, 0.1 percent CPP-ACP prevented mineral loss and provided a potential source of calcium for subsequent remineralization, thus restricting the caries process.

In 2005, Ramalingam and colleagues[29] immersed human enamel specimens in an erosive sports drink (Powerade [Coca-Cola, Atlanta] alone, Powerade with four concentrations of CPP-ACP [0.063, 0.09, 0.125 and 0.25 percent] and double deionized water as the placebo). Scanning electron microscopic examination of the specimens showed that the erosive lesions that developed in specimens immersed in Powerade were eliminated with the addition of CPP-ACP at all concentrations except 0.063 percent. The taste panel could not distinguish Powerade from Powerade with 0.125 percent CPP-ACP. The authors concluded that adding CPP-ACP to the sports drinks significantly reduced the beverage's erosivity without affecting the product's taste.

However, in another in vitro study, Lennon and colleagues[30] applied a tooth cream containing 5 percent casein/calcium phosphate to bovine enamel specimens for 120 seconds twice daily. They found no significant difference with respect to erosive enamel loss (bovine enamel specimens rinsed with artificial saliva interrupted by 1 percent citric acid (pH 2.3) for 30 seconds six times daily for 14 days) when compared with the no-treatment control specimens after seven and 14 days of erosive cycling.

Clinical picture. According to the manufacturer (GC America),[31] CPP-ACP is a useful cariostatic agent for the control of dental caries, and it can be used as an adjunct preventive therapy to reduce caries in high-risk patients, to reduce dental erosion in patients with gastric reflux or other disorders, to reduce decalcification in orthodontic patients, to repair enamel in cases involving white-spot lesions, orthodontic decalcification or fluorosis or before and after tooth whitening) and to desensitize teeth (for example, reducing hypersensitivity resulting from whitening procedures, treating sensitive dentin in patients with dental erosion and reducing sensitivity resulting from exposed root surfaces after professional tooth cleaning).

One advantage of this therapy is that the products (MI Paste, Tooth Mousse, Trident White Gum) are ingestible. In contrast, topical fluoride therapy poses a risk if the patient ingests a significant amount of fluoride.[32] However, for any clinical application, clinicians should consider potential side effects from ingestion of casein derivative protein in people with immunoglobulin E allergies to milk proteins. We should note that CPP-ACP is digestible by people with lactose intolerance.

MI Paste Plus is a recently introduced product that contains 900 ppm fluoride. Although it is designed to increase enamel remineralization through the deposition of fluoride-containing calcium-phosphate precipitates, no reports, to our knowledge, have been published that demonstrate its efficacy in vivo. Furthermore, at 900 ppm fluoride, this product is not considered ingestible and, therefore, children younger than 6 years should not use it. Moreover, fluoride potentially can interact with the ACP component of the casein complex and may precipitate out as calcium fluoride, rendering both inorganic components ineffective. Development of the carrier for calcium and phosphate in the Recaldent technology (as casein in MI Paste Plus) has greatly reduced this problem. However, independent research is required to study the interaction of the fluoride-enriched CPP-ACP complex with enamel.

Three main calcium phosphate products have been tested or are on the market to inhibit or reverse caries:

- products composed of inorganic calcium phosphate minerals alone[33] (Enamel Pro Prophy Paste, Premier Products, Plymouth Meeting, Pa.);
- products composed of inorganic calcium phosphate minerals plus silica (NovaMin, NovaMin Technology, Alachua, Fla.);
- products composed of the CPP-ACP complex.

To our knowledge, no systematic reviews of the clinical trials of these products have been conducted to date. Therefore, the purpose of this report is to systematically review the clinical trials of one of these types of products used in dentistry: products composed of the CPP-ACP complex.

METHODS

Data sources. We conducted a comprehensive literature search of databases Ovid MEDLINE, Cumulative Index to Nursing & Allied Health Literature, Evidence Based Medicine of Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Database of

TABLE 1

Literature search strategy.

SEARCH HISTORY AND CRITERIA	NO. OF ARTICLES
Key Words: "MI Paste," "Recaldent," "casein phosphopeptides-amorphous calcium phosphate," "casein phosphopeptide-amorphous calcium phosphate," "CPP-ACP" or "Tooth Mousse"	98
Duplicate articles removed	58
Search limited to humans	54
Search limited to English-language articles	53
Relevant articles at title stage (agreement between reviewers: 93 percent)	16
Relevant articles at abstract stage (agreement between reviewers: 100 percent)	11
Relevant articles at full copy stage (agreement between reviewers: 100 percent)	11
Article suggested by reviewers of The Journal of the American Dental Association	1
Articles scored and included in evidence tables	12

Abstracts of Reviews of Effects, EMBASE, Health and Psychosocial Instruments, HealthSTAR/Ovid Healthstar and International Pharmaceutical Abstracts for any published and unpublished studies (from their inception until October 2007). We also searched the Web sites of product manufacturers ("www.gcamerica.com", "www.recaldent.com"), as well as Google Scholar. Table 1 shows the key words and their combinations used in the literature search.

Inclusion and exclusion criteria. We included randomized and quasi-randomized controlled trials of the efficacy of casein derivatives in any clinical dental application. We excluded in vitro studies, case series, case reports, letters to editors (not containing primary data), editorials, review articles and commentaries, but we read them to identify any potential studies.

Study selection, data extraction and quality assessment. We retrieved 98 articles. After removing duplicates, we limited the searches to articles in English and to studies that involved human subjects. We selected 53 citations and searched for relevance (determined by title), which resulted in 16 articles. We reviewed the abstracts of these articles for relevance, which resulted in 12 articles that we selected for retrieval and copying. Both of us reviewed all articles at each stage independently and we resolved discrepancies by consensus. One of us (A.A.) printed the abstraction sheets for annotated references (that is, with citation, author/date, population, age, sex, geographic location, intervention or test treatment (number of subjects), control treatment (number of subjects), outcome, critical appraisal comments, conclusion, strength of evidence and classification of recommendations. We then read and reviewed the abstraction sheets to determine relevance and scored them to rate the evidence for this review. In reading the articles, we checked the reference lists to identify any other articles that may have been relevant to the research question or provided additional information. All of these were found in the original searches.

Best available evidence. We summarized the best available evidence by using inclusion criteria and measuring the strength and quality of the studies according to the evidence classification system developed by the Canadian Task Force on Preventive Health Care[34] (Tables 2 and 3). This system includes a hierarchy of evidence from the highest (level I)—a properly randomized controlled trial—to the lowest (level III)—opinions of respected authorities, based on clinical experience, descriptive studies or reports of expert committees. This system also includes a bidirectional classification of its recommendations (that is, strength of the recommendation for or against specific clinical preventive actions).

We critically reviewed the retrieved articles using the checklist for appraising evidence in health care.[35] The checklist consists of questions addressing ethics, study design, methodology and appropriateness of the results for the population of interest.

SUMMARY OF EVIDENCE AND COMPARISON OF OUTCOMES

We found 12 studies of the efficacy of casein derivatives in clinical dentistry[10,11,36-45] (Table 4, page 920). The outcomes of interest were caries prevention (10 studies[10,11,36-43]), relief from dry-mouth symptoms (one study[44]) and treating dentin hypersensitivity (one study[45]).

Caries prevention. This review identified 10 studies of caries prevention via treatment with casein derivatives. Eight of these studies[10,11,36,38-42] were randomized clinical trials with crossover designs that determined the remineralizing effects of CPP-ACP by using in situ caries models.

In all but one of these studies,[41] subjects wore custom-made removable midpalatal acrylic appliances that covered the first premolars to the last tooth in the arch and were retained by four stainless steel circumferential clasps. These appliances contained troughs, each of which housed two or three demineralized enamel half-slabs by means of wax retention. In all but one study, sound human extracted third molars were the source of the enamel. In one study,[41] bovine enamel sections were used.

To create the demineralized lesion, the investigators polished sound enamel, covered it with an acid-resistant coating (nail varnish or epoxy resin) and then subjected the enamel to demineralizing solutions. This procedure produced consistent subsurface lesions of 80 to 110 micrometers in depth.

At the end of each treatment period (that is, after subjects consumed sugar-free gum, lozenge, milk or mouthrinse containing CPP-ACP), the investigators took the remineralized enamel half-slabs and their paired demineralized control half-slabs (retained in a humidified environment) and embedded and sectioned them. Then they subjected the slabs to microradiography and computer-assisted microdensitometric analysis to evaluate the mean subsurface lesion depth, percentage of subsurface remineralization and/or change in mineral profile.

For each test group, the length of study varied from seven to 21 days. Only one study[39] had a washout period of four weeks; the rest had a washout period of five to seven days. Overall, all of the studies except one[41] showed that CPP-ACP had caries-preventive potential and resulted in subsurface remineralization of the enamel with the in situ carious lesion in a dose-response fashion.

The only study that did not show a difference between the CPP-ACP–containing chewing gums and the control chewing gums was a 2007 randomized crossover in situ study.[41] While investigators in the other studies placed enamel slabs at the palate of midpalatal appliances, subjects in the study by Schirrmeister and colleagues[41] wore custom-made removable buccal appliances in the mandible; these appliances had a buccal resin wing on each side, and two bovine enamel specimens were mounted in each wing flush with the buccal surface. This may have resulted in less direct contact between the slabs and the chewing gums.

TABLE 2

Canadian Task Force grades of recommendation for specific clinical preventive actions.*

GRADE	EVIDENCE
A	Good evidence to recommend the clinical preventive action
B	Fair evidence to recommend the clinical preventive action
C	The existing evidence is conflicting and does not allow making a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making
D	Fair evidence to recommend against the clinical preventive action
E	Good evidence to recommend against the clinical preventive action
I	Insufficient evidence (in quantity and/or quality) to make a recommendation; however, other factors may influence decision-making

* Adapted with permission of the Canadian Task Force on Preventive Health Care.[34]

TABLE 3

Levels of evidence.*

LEVEL	TYPE OF EVIDENCE
I	Evidence from randomized controlled trials
II-1	Evidence from controlled trials without randomization
II-2	Evidence from cohort or case-control analytic studies, preferably from more than one center or research group
II-3	Evidence from comparisons between times or places with or without the intervention; dramatic results in uncontrolled experiments could be included here
III	Opinions of respected authorities, based on clinical experience; descriptive studies or reports of expert committees

* Adapted with permission of the Canadian Task Force on Preventive Health Care.[34]

The other two studies[37,43] in the caries-prevention category were in vivo (rather than in situ) studies. The first study[37] was a randomized clinical trial comparing a mouthrinse that contained casein derivatives coupled with calcium phosphate (CD-CP) with a sodium fluoride (NaF) mouthrinse. Subjects with salivary gland dysfunction (resulting from radiotherapy for head and neck cancer or Sjögren syndrome) used one of the two mouthrinses three times per day for one year. The investigators measured the coronal caries increment with posterior bitewing radiographs

TABLE 4

Evidence of clinical efficacy of casein derivatives.

AUTHOR, YEAR	POPULATION	INTERVENTION	CONTROLS	OUTCOME	STUDY AUTHORS' CONCLUSIONS	COMMENTS
Andersson and colleagues, 2007[43]	26 adolescents (60 teeth, 152 visible WSLs*; mean age, 14.6 years)	13 subjects, 70 WSLs; CPP-ACP† paste daily for 3 months, then fluoride paste daily for 3 months	13 controls, 62 WSLs; daily 0.05% NaF‡ mouthrinse and fluoride paste for 6 months	Blind assessment of clinical and laser fluorescence scores of WSLs at 1, 3, 6 and 12 months	Both treatments reversed WSLs; better visual outcome for test	Single-blinded RCT,§ small sample, no power calculation; subjective scoring; no significant difference with laser fluorescence
Cai and colleagues, 2007[42]	10 subjects (age, 23-46 years)	In situ: 3 sugar-free gums: 20 mg¶ citric acid and 18.8 mg CPP-ACP; 20 mg citric acid; no added ingredient	Crossover; washout 1 week	% subsurface remineralization	Significantly greater ($P < .05$) mineral level after acid challenge with CPP-ACP	Double-blinded, randomized, crossover, in situ
Schirrmeister and colleagues, 2007[41]	15 subjects (mean ± SD# age, 27.5 ± 2.5 years)	In situ: 4 sugar-free gums: without zinc citrate; with zinc citrate and dicalcium phosphate, calcium gluconate, calcium lactate; with CPP-ACP; no calcium	Chewed test gums 14 days each; for fifth period (control), subjects wore appliances without chewing gum	Lesion depth reduction and mineral change	No additional remineralizing benefit even with gum and CPP-ACP	Randomized, crossover, in situ; observer blinded; mandibular buccal appliances may have resulted in less direct contact of slabs with gums
Walker and colleagues, 2006[40]	10 adults	In situ: subjects drank 200 mL** control milk or test milk with 2 or 5 grams CPP-ACP/L††	Crossover; washout 1 week	% subsurface remineralization	More remineralizing ability for milk and CPP-ACP	Double-blinded RCT, crossover, short washout, small sample size
Reynolds and colleagues, 2003[39]	30 adults (age, 22-44 years)	Mouthrinse: 2% CPP-ACP, 6% CPP-ACP, calcium and phosphate mixture, deionized water; sugar-free gum: in situ; $CaCO_3$,‡‡ $CaHPO_4/CaCO_3$§§ or CPP-ACP	Crossover; washout 4 weeks for mouthrinse; not noted for gum	Mouthrinse: plaque calcium and inorganic phosphate levels; gum: % subsurface remineralization	Importance of CPP in delivering ACP to tooth surface and stabilizing ACP	Double-blinded RCT, crossover, in situ
Iijima and colleagues, 2004[10]	10 adults (mean ± SD age, 32.3 ± 7.9 years)	In situ: sugar-free gum containing 18.8 mg CPP-ACP	Control; sugar-free gum lacking CPP-ACP; crossover; washout 1 week	% subsurface remineralization	Sugar-free gum and CPP-ACP effective in remineralization	Double-blinded RCT, crossover, short washout, small sample size
Cai and colleagues, 2003[36]	10 adults (mean ± SD age, 34 ± 6.6 years)	In situ: 3 lozenge types with CPP-ACP (0%, 1% and 3% weight per weight ratio)	No lozenge	% subsurface remineralization	Lozenges may be suitable for delivery of CPP-ACP to promote enamel remineralization (dose-related)	Double-blinded RCT, crossover, short washout, small sample size

obtained at baseline and at the 12-month follow-up visit. The results showed no difference between the NaF mouthrinse and the CD-CP mouthrinse with regard to caries preventive efficacy.

The other study[43] was a single-blinded

TABLE 4 (CONTINUED)

AUTHOR, YEAR	POPULATION	INTERVENTION	CONTROLS	OUTCOME	STUDY AUTHORS' CONCLUSIONS	COMMENTS
Shen and colleagues, 2001[11]	30 adults (age, 23-40 years)	In situ: no treatment group; gum and different CPP-ACP mg (0, 0.19, 10, 18.8, 56.4)	Crossover; washout 1 week	% subsurface remineralization	Dose-related increase in remineralization with CPP-ACP and sorbitol- or xylitol-based sugar-free gum	Double-blinded RCT, crossover, short washout
Hay and Thomson 2002[37]	124 subjects with salivary gland dysfunction (mean ± SD age, 53 ± 14 years)	N = 63; self-administered topical CD-CP¶¶ mouthrinse 3 times daily	N = 61: self-administered topical 0.05% NaF mouthrinse 3 times daily	Coronal caries increment (bitewing radiographs at baseline and 12 months	CD-CP may be useful for caries prevention in dry mouth syndrome	RCT (double-blinding unclear); small sample for 90% power (type II error); no control over care outside study
Itthagarun and colleagues, 2005[38]	12 adults (age, 20-47 years)	In situ: gum with 30 mg urea and no calcium phosphate, 25 mg dicalcium phosphate dehydrate or 47 mg CPP-ACP	Crossover; washout 5 days	Mean % change in lesion depth	Caries-preventive potential of urea-containing gum and dicalcium phosphate or CPP-ACP	Double-blinded RCT, crossover, short washout, subjects supplied with cariogenic snack food
Kowalczyk and colleagues, 2006[45]	101 teeth with dentin hypersensitivity in 13 patients (age, 23-48 years)	GC Tooth Mousse## applied on surfaces for 3 minutes	None	Pain intensity at baseline; testing pain intensity soon after applying GC Tooth Mousse, and at 15 minutes, 1 week and 4 weeks after application	Insufficient effectiveness and short-term therapeutic effect in soothing pain	Uncontrolled cohort; 58% follow-up loss; no blinding; no control over outside care; tooth-level analysis, not subject; remineralizing potential unknown
Hay and Morton, 2003[44]	38 adults with severe xerostomia (age, older than 25 years)	CD-CP for 14 days	Patients' mouth-moistening strategies (sipping water, chewing gum, artificial saliva)	Questionnaire about benefit	Potential benefits of CD-CP mouthrinse in oral moistening and dental caries prevention in xerostomia	Descriptive; self-evaluation questionnaire; caries-prevention potential cannot be concluded; control group not good

* WSLs: White-spot lesions.
† CPP-ACP: Casein phosphopeptide—amorphous calcium phosphate.
‡ NaF: Sodium fluoride.
§ RCT: Randomized controlled trial.
¶ mg: Milligrams.
# SD: Standard deviation.
** mL: Milliliters.
†† L: Liter.
‡‡ $CaCO_3$: Calcium carbonate.
§§ $CaHPO_4/CaCO_3$: Calcium hydrogen phosphate/calcium carbonate.
¶¶ CD-CP: Casein derivatives coupled with calcium phosphate.
GC Tooth Mousse is manufactured by GC Europe N.V., Leuven, Belgium.

randomized clinical trial of 26 healthy adolescents with 152 visible white-spot lesions on 60 incisors and canines. Immediately after undergoing debonding of fixed orthodontic appliances and receiving a professional cleaning, subjects were randomly assigned to two groups. The test group (n = 13 subjects with 70 sites) applied a topical dental cream containing CPP-ACP (Topacal C-5, Nulite Systems International, Hornsby, Australia) daily for three months fol-

lowed by a three-month regimen of daily toothbrushing with a fluoridated dentifrice. The control group (n = 13 subjects with 62 sites) rinsed daily with a 0.05 percent sodium fluoride mouthwash and used fluoridated dentifrice for six months.

The results showed a significant improvement (regression) in white-spot lesions within each group across a 12-month follow-up period with the use of clinical assessment of the lesions (visual scoring on a scale from 0 to 4, with 0 being no visible color change and 4 being a distinct white color change) and laser fluorescence reading (DIAGNOdent, KaVo, Biberach, Germany). Moreover, the clinical visual scoring showed significant improvements ($P < .01$) for the group that underwent the CPP-ACP regimen with regard to the number of sites that disappeared completely after three months (55 percent in the test group versus 18 percent in the control group) and after 12 months (63 percent in the test group versus 25 percent in the control group). However, the study results showed no significant differences between the groups—at baseline or at any of the follow-up visits—when the investigators considered the laser fluorescence measurements.

Treating dry mouth. As an extension to the study by Hay and Thomson,[37] Hay and Morton[44] administered a self-evaluation survey to 38 patients in the original sample. The survey asked them to compare the CD-CP mouthrinse with their usual mouth-moistening strategies (for example, sipping water, chewing gum, using artificial saliva). The authors concluded that the CD-CP mouthrinse, when used as an atomized spray in the mouth, provided good moistening and lubrication. However, some methodological flaws (Table 2) limited the level of evidence and strength of the recommendation.

Treating dentin hypersensitivity. We identified one prospective study[45] that evaluated the efficacy of CPP-ACP (GC Tooth Mousse) in the treatment of patients with dentin hypersensitivity. The study concluded that the efficacy and short-term therapeutic effect of CPP-ACP were insufficient in treating dentin hypersensitivity. However, the study lacked an appropriate control group and masking of the evaluators.

The authors found insufficient clinical trial evidence to make a recommendation regarding the long-term effectiveness of casein derivatives in preventing caries in vivo.

EVIDENCE-BASED RECOMMENDATIONS

This report aimed to review the clinical trials of casein derivatives used in dentistry. We identified 10 studies (eight in situ and two in vivo) that focused on caries prevention. Seven of these studies showed that casein derivatives (both CD-CP and CPP-ACP, but specifically CPP-ACP) are efficacious in preventing dental caries, while two did not find any additional caries-preventive effect of casein derivatives when comparing the test groups with control groups. The results of the last study[43] were conflicting. These studies were conducted in Australia (six studies[10,11,36,39,40,42]), New Zealand (one study,[37] which included a descriptive survey regarding patient satisfaction[44]), Hong Kong (one study[38]), Germany (one study[41]) and Sweden (one study[43]). We also identified one prospective study conducted in Poland[45] that found insufficient effectiveness and short-term therapeutic effect of CPP-ACP (GC Tooth Mousse) in treating dentin hypersensitivity. In appraising the evidence, we point out the following.

Investigators. First, six of the eight in situ studies were conducted by the same group of investigators who patented the CPP-ACP complex.[10,11,36,39,40,42] These six studies, as well as another study,[38] followed the same in situ protocol that resulted in significant findings in favor of this technology.

Findings. Second, of the four studies conducted by groups independent of those that patented the complex, one[38] used a similar in situ model. Although the findings point to significant caries preventive potential of CPP-ACP when added to urea-containing chewing gum, the study[38] found no difference in outcomes between CPP-ACP and dicalcium phosphate dihydrate. Consequently, the results of this study[38] do not support the conclusion that the CPP-ACP molecule consists of any special properties in comparison with dicalcium phosphate dehydrate.

The other independent study[41] was conducted in Germany using a different in situ model, as described earlier. This study found no significant differences between chewing gums that contained or did not contain calcium with regard to both mineral change and depth of demineralized

lesions. The third independent study[37] was an in vivo trial that assessed carious lesions in patients with salivary gland dysfunction. The results showed no difference between a NaF mouthrinse and a CD-CP mouthrinse. However, the number of teeth lost was significantly higher in the CD-CP group. We should note that the population recruited for this study was at high risk of experiencing tooth loss and dental caries; thus, the results may not be generalizable to a general population.

The results of the last independent study,[43] which was conducted in Sweden, were conflicting. Using a clinical visual scoring system, the researchers found significant regression of white spot lesions (resulting from one year of fixed orthodontic treatment) in the CPP-ACP group compared with the control group (which received 0.05 percent NaF mouthrinse daily). However, when analyzing the laser fluorescence measurements, the authors found no significant differences between the groups over time.

Third, a limitation of the in situ methodological approach, however, is that long-term caries prevention has yet to be fully explored. In vivo clinical trials with adequate follow-up are needed to determine the efficacy of CPP-ACP in caries prevention.

Finally, none of the studies tested for the potential formation of calculus resulting from the supersaturated calcium phosphate state in plaque.

CONCLUSION

We conclude that there is insufficient clinical trial evidence (in quantity, quality or both) to make a recommendation regarding the long-term effectiveness of casein derivatives, specifically CPP-ACP, in preventing caries in vivo and in treating dentin hypersensitivity or dry mouth. The highest level of evidence—that is, well-designed and -conducted, double-blind, randomized clinical trials with adequate sample size, limited or no loss to follow up and carefully standardized methods of measurement and analysis—is needed to enable researchers to evaluate the efficacy and cost-effectiveness of casein derivatives in various products (for example, chewing gum versus dental paste, lozenges, mouthrinse) in reducing or eliminating dental caries, white-spot lesions or dentin hypersensitivity. This is especially important in clinical trials in which outcomes are measured in vivo. ■

Disclosure. Drs. Azarpazhooh and Limeback did not report any disclosures.

For a complete version of Table 4, see supplemental data in the online version of this article at "http://jada.ada.org".

The authors thank the JADA reviewers for their time, patience and constructive comments and suggestions to improve the manuscript.

1. Aimutis WR. Bioactive properties of milk proteins with particular focus on anticariogenesis. J Nutr 2004;134(4):989S-995S.
2. Guggenheim B, Schmid R, Aeschlimann JM, Berrocal R, Neeser JR. Powdered milk micellar casein prevents oral colonization by *Streptococcus sobrinus* and dental caries in rats: a basis for the caries-protective effect of dairy products. Caries Res 1999;33(6):446-454.
3. Krobicka A, Bowen WH, Pearson S, Young DA. The effects of cheese snacks on caries in desalivated rats. J Dent Res 1987;66(6):1116-1119.
4. Reynolds EC, del Rio A. Effect of casein and whey-protein solutions on caries experience and feeding patterns of the rat. Arch Oral Biol 1984;29(11):927-933.
5. Rosen S, Min DB, Harper DS, Harper WJ, Beck EX, Beck FM. Effect of cheese, with and without sucrose, on dental caries and recovery of *Streptococcus mutans* in rats. J Dent Res 1984;63(6):894-896.
6. Harper DS, Osborn JC, Clayton R, Hefferren JJ. Modification of food cariogenicity in rats by mineral-rich concentrates from milk. J Dent Res 1987;66(1):42-45.
7. Silva MF, Burgess RC, Sandham HJ, Jenkins GN. Effects of water-soluble components of cheese on experimental caries in humans. J Dent Res 1987;66(1):38-41.
8. Reynolds EC. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. J Dent Res 1987;66(6):1120-1127.
9. Schupbach P, Neeser JR, Golliard M, Rouvet M, Guggenheim B. Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci. J Dent Res 1996;75(10):1779-1788.
10. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res 2004;38(6):551-556.
11. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001;80(12):2066-2070.
12. Reynolds EC, Cain CJ, Webber FL, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. J Dent Res 1995;74(6):1272-1279.
13. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist 1998;18(1):8-16.
14. Huq NL, Cross KJ, Reynolds EC. Molecular modelling of the multiphosphorylated casein phosphopeptide alphaS1-casein(59-79) based on NMR constraints. J Dairy Res 2004;71(1):28-32.
15. Mathew M, Takagi S. Structures of biological minerals in dental research. J Res Natl Inst Stand Technol 2001;106:1035-1044.
16. Office of Premarket Approval, Center for Food Safety and Applied Nutrition. Agency response letter GRAS notice no. GRN 000011. Rockville, Md.: U.S. Food and Drug Administration; 1999. "www.webcitation.org/5SfNOmXWG". Accessed June 9, 2008.
17. Office of Device Evaluation, Center for Devices and Radiological Health. 510(k) premarket notification database. Rockville, Md.: U.S. Food and Drug Administration; 2007. "www.webcitation.org/5SfOiUhEu". Accessed Oct. 17, 2007.
18. Bavetta LA, McClure FJ. Protein factors and experimental rat caries. J Nutr 1957;63(1):107-117.
19. Reynolds EC, Black CL. Confectionery composition and rat caries. Caries Res 1987;21(6):538-545.
20. Reynolds EC, Black CL. Cariogenicity of a confection supplemented with sodium caseinate at a palatable level. Caries Res 1989;23(5):368-370.
21. Schweigert B, Shaw J, Zepplin M, Elvehjem C. Dental caries in the cotton rat, part VI: the effect of the amount of protein, fat, and carbohydrate in the diet on the incidence and extent of carious lesions. J Nutr 1946;31:439-447.
22. Schweigert B, Potts E, Shaw J, Zepplin M, Phillips P. Dental caries in the cotton rat, part VIII: further studies on the dietary effects of carbohydrate, protein, and fat on the incidence and extent of carious

lesions. J Nutr 1946;32:405-412.
23. Shaw JH. Effects of dietary composition on tooth decay in the albino rat. J Nutr 1950;41(1):13-24.
24. Reynolds EC, Black CL, Cai F, et al. Advances in enamel remineralization: anticariogenic casein phosphopeptide-amorphous calcium phosphate. J Clin Dent 1999;10:86-88.
25. Reynolds EC, Cain CJ, Webber FL, et al. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptides in the rat. J Dent Res 1995;74(6):1272-1279.
26. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76(9):1587-1595.
27. Rose RK. Binding characteristics of *Streptococcus mutans* for calcium and casein phosphopeptide. Caries Res 2000;34(5):427-431.
28. Rose RK. Effects of an anticariogenic casein phosphopeptide on calcium diffusion in streptococcal model dental plaques. Arch Oral Biol 2000;45(7):569-575.
29. Ramalingam L, Messer LB, Reynolds EC. Adding casein phosphopeptide-amorphous calcium phosphate to sports drinks to eliminate in vitro erosion. Pediatr Dent 2005;27(1):61-67.
30. Lennon AM, Pfeffer M, Buchalla W, Becker K, Lennon S, Attin T. Effect of a casein/calcium phosphate-containing tooth cream and fluoride on enamel erosion in vitro. Caries Res 2006;40(2):154-157.
31. Prospec MI Paste. Alsip, Ill.: GC America. "www.gcamerica.com/images/pdfs/mi_clinical_bro.pdf". Accessed June 4, 2008.
32. Hawkins R, Locker D, Noble J, Kay EJ. Prevention, part 7: professionally applied topical fluorides for caries prevention. Br Dent J 2003;195(6):313-317.
33. Garvin J. ACP: the next big thing. "www.ada.org/prof/resources/pubs/adanews/adanewsarticle.asp?articleid=2632". Accessed June 4, 2008.
34. Canadian Task Force on Preventive Health Care. Methodology. Ottawa, Ontario: Health Canada; 1994. "www.webcitation.org/5SfPwrRYq". Accessed May 22, 2008.
35. Azarpazhooh A, Mayhall JT, Leake JL. Introducing dental students to evidence-based decisions in dental care. J Dent Educ 2008;72(1):87-109.
36. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aust Dent J 2003;48(4):240-243.
37. Hay KD, Thomson WM. A clinical trial of the anticaries efficacy of casein derivatives complexed with calcium phosphate in patients with salivary gland dysfunction. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93(3):271-275.
38. Itthagarun A, King NM, Yiu C, Dawes C. The effect of chewing gums containing calcium phosphates on the remineralization of artificial caries-like lesions in situ. Caries Res 2005;39(3):251-254.
39. 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. J Dent Res 2003;82(3):206-211.
40. Walker G, Cai F, Shen P, et al. Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. J Dairy Res 2006;73(1):74-78.
41. Schirrmeister JF, Seger RK, Altenburger MJ, Lussi A, Hellwig E. Effects of various forms of calcium added to chewing gum on initial enamel carious lesions in situ (published correction in Caries Res 2007;41[3]:243). Caries Res 2007;41(2):108-114.
42. Cai F, Manton DJ, Shen P, et al. Effect of addition of citric acid and casein phosphopeptide-amorphous calcium phosphate to a sugar-free chewing gum on enamel remineralization in situ. Caries Res 2007;41(5):377-383.
43. 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 Prev Dent 2007;5(3):229-233.
44. Hay KD, Morton RP. The efficacy of casein phosphoprotein-calcium phosphate complex (DC-CP) [Dentacal] as a mouth moistener in patients with severe xerostomia. N Z Dent J 2003;99(2):46-48.
45. Kowalczyk A, Botulinski B, Jaworska M, Kierklo A, Pawinska M, Dabrowska E. Evaluation of the product based on Recaldent technology in the treatment of dentin hypersensitivity. Adv Med Sci 2006;51 (suppl 1):40-42.