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Effect of Conventional and Acid-modified Casein Phosphopeptide-Amorphous Calcium Phosphate Crèmes on Dentin Permeability Before and After Acid Challenge

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Clinical Relevance

In vitro application of CPP-ACP–containing crèmes for two weeks has a tendency to reduce dentin hypersensitivity by occluding dentinal tubules.

SUMMARY

This study investigated the effect of conventional and acidified casein phosphopeptide-amorphous calcium phosphate–containing crèmes (CPP-ACP and ACPP-ACP) on dentin permeability (DP) before and after acid challenge, using a fluid-flow measuring device and scanning electron microscopic (SEM) examination. Fifty dentin discs were prepared from intact human third molars. Each tooth was sectioned 3 mm above and below the cemento-enamel junction. The smear layer was removed to expose the dentin and dentinal tubules. For fluid-flow measurement, 20 specimens were used and divided into two groups: (a) CPP-ACP (pH=7) and (b) ACPP-ACP (pH=2). The dentin surfaces were treated with the crème for five minutes daily for two weeks, and then an acid challenge was performed using 6% citric acid. Under simulated pulpal pressure, DP was measured at baseline, five minutes, two weeks, and after acid challenge. The remaining specimens were prepared and ex-

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amined using SEM to investigate dentinal tubule occlusion. DP was not significantly different between the two CPP-ACP–containing crèmes at any test period ($p \geq 0.05$). DP after two weeks or acid challenge was significantly lower than that at five minutes ($p < 0.05$). The results were consistent with the SEM micrographs that presented patent dentinal tubules after a five-minute application and partial blockage of dentinal tubules after the two-week application. The dentinal tubules remained partially occluded after acid challenge. Using conventional and acid-modified CPP-ACP–containing crèmes for two weeks decreased DP and partially occluded the dentinal tubules, which resisted acid challenge.

INTRODUCTION

Dentin hypersensitivity is characterized by short, sharp pain arising from exposed dentin in response to thermal, evaporative, tactile, osmotic, or chemical stimuli.[1] According to the hydrodynamic theory, dentinal fluid movement within dentinal tubules of exposed dentin is responsible for dentin hypersensitivity.[2]

Hypersensitive dentin can be conservatively treated by using a desensitizing agent to either block neural transmission or occlude the dentinal tubules.[3] In the former, a common desensitizing agent blocking neural transmission is potassium nitrate or potassium chloride.[4] In the latter, several desensitizing products with the action of occluding dentinal tubules, such as glutaraldehyde, oxalate, fluoride, or resin-based bonding agents, have been introduced.[5,6] Application of dental lasers may also reduce dentin hypersensitivity by occluding dentinal tubules, coagulating proteins in the dentinal fluid, or altering nerve activity.[7] Using a laboratory-based dentin disc model measuring dentin permeability (DP) to evaluate the potential of a desensitizing agent or dental laser is a useful screening method.[8]

Casein phosphopeptide-amorphous calcium phosphate–containing crème (CPP-ACP) is commonly used for remineralization of initial carious enamel lesions.[9] CPP-ACP can also be used as a desensitizing agent in reducing dentin hypersensitivity.[10] The mechanism of CPP-ACP in reducing dentin hypersensitivity is possibly due to its remineralizing action[11] and precipitation as hydroxyapatite, which most likely leads to occlusion of the dentinal tubules. Duration of use would be an important factor since a longer application period might increase the precipitations for desensitization.[12] For this reason, a one- to two-week application period has been suggested.

Improvement of CPP-ACP for desensitizing hypersensitive dentin is possible. A calcium phosphate solution has the highest precipitation rate in acidic conditions at a pH of about 2.[13] CPP-ACP is likely to be precipitated in the form of a calcium phosphate salt and is effective in reducing dentin hypersensitivity if modified with an acid. It might be assumed that the ability to create a precipitate to occlude dentinal tubules tends to increase when a CPP-ACP–containing crème is modified with an acid.

The desensitizing effect, however, may not be permanent in the oral environment since acidic diets or drinks could dissolve desensitizing products and reopen the dentinal tubules.[6] Thus, a successful desensitizing agent should withstand acid challenge. Citric acid at 6% has usually been used in laboratory-based studies to test acid resistance of desensitizing agents.[6]

The purpose of this study was to investigate the effect of CPP-ACP–containing crème and an acid-modified CPP-ACP crème (ACPP-ACP) on DP before and after acid challenge in a dentin disc model, using a fluid-flow measuring device and scanning electron microscopic (SEM) examination.

METHODS AND MATERIALS

Fifty intact human mandibular third molars were collected, stored in a 0.12% thymol solution, and used within a week. The roots of the teeth were sectioned 3 mm above and below the cementoenamel junction using a diamond blade with water coolant (Isomet, Buehler Ltd, Lake Bluff, IL, USA). Pulp tissue remnants were removed with tweezers. The pulpal side was etched with 37% phosphoric acid (ScotchBond Etchant, 3M ESPE, St Paul, MN, USA) for 15 seconds and rinsed with normal saline.

Each sectioned dentin surface was sequentially polished under running water with 600- to 4000-grit silicon carbide papers to remove remnants of enamel and achieve a thin smear layer. The smear layer and smear plugs were removed with 17% EDTA solution (Endo Clean, M-Dent, Bangkok, Thailand) for 60 seconds to simulate exposed dentin with patent dentinal tubules. The dentin surfaces were photographed with a grid of 1 mm^2 under a polarized light microscope at 40× magnification; a dentin surface area was located and calculated into mm^2. The specimens were kept in 0.9% sodium chloride (normal saline) solution before testing.

Dentin Permeability Measurement

Each dentin disc was mounted on a Perspex plate perforated with a 21-gauge needle bonded with cyanoacrylate adhesive. The attached area was subsequently covered with epoxy resin. The pulp chamber of the specimen was filled with normal saline and attached to a DP-measuring device (Mahidol University model, Bangkok, Thailand). DP was measured by detecting fluid by distance within a capillary tube. In the model, simulated pulpal pressure was set at a 14-cm height throughout the experiment.[12] The exposed occlusal dentin surface was saturated with normal saline and covered with a humidified cup to prevent evaporation during fluid flow measurement. To check for any leakage in the system, the device was connected to a root-sectioned tooth with intact coronal structure for 10 minutes, and the absence of fluid movement in the system was confirmed. Fluid movement through untreated dentin was recorded for five minutes and recorded as the baseline DP.

Twenty dentin discs were randomly selected and distributed into two groups (n=10) in each group; the dentin surfaces were treated with either CPP-ACP–containing crèmes (Tooth Mousse, GC Corp, Itabashi-Ku, Tokyo, Japan) or ACPP-ACP. The latter was prepared by mixing CPP-ACP with 2 mol/L phosphoric acid solution (pH=1.15), at a ratio of 1:1 by volume and then used immediately after mixing. The paste was applied on a moist dentin surface (agitating with an applicator) for one minute, left undisturbed for four minutes and then removed with a moist cotton swab. Fluid movement was recorded for five minutes as the *5-min DP*.

The specimen was removed from the device and kept in normal saline solution at 37°C for 24 hours. The application procedure was repeated every day for two weeks. Each specimen was reattached to the device, and fluid movement was remeasured for five minutes, as the *2-week DP*. After that, the dentin surfaces were treated with 6% citric acid for five minutes and rinsed with normal saline to remove the acid. Fluid movement was recorded for five minutes after acid challenge, as the *acid-challenge DP*.

DP was calculated from the amount of fluid movement within the capillary tube. Fluid movement, in millimeters, was converted into volume (V) by using the formula, $V(\mu L) = \pi r^2 \times d$, where r = radius of the capillary tube (150 μm) and d = distance of fluid movement in μm. DP was then calculated from the formula, DP ($\mu L/s/mm^2/mm\ H_2O$) = $V/(T \times A \times P)$, where T = time in seconds, A = area of dentin in mm^2, and P = simulated pressure in mm H_2O. Compared with the baseline value, the percentage change in DP was calculated from the formula, $DP_P - DP_B/DP_B \times 100$ (DP_P=DP at a measuring period; DP_B=P at baseline). For percentage change in DP, a negative value (–) indicated a decreased DP while a positive value (+) meant an increased DP.

Scanning Electron Microscopic Examination

Thirty dentin discs were prepared as previously described. Eighteen discs were randomly divided into two groups according to the two CPP-ACP crèmes, nine of each. The specimens in each group were further divided into three subgroups following the three observation periods: five-minute and two-week applications and after acid challenge (n=3 per subgroup). Three untreated discs were used as the control. In addition, CPP-ACP–containing crèmes were diluted with distilled water at a ratio of 1:1 by volume and applied on nine dentin discs, three for each observation period, to observe the dilution effect of CPP-ACP on precipitation. The dentin surfaces were prepared and examined using SEM at a magnification of 2000×.

The dentin discs were dried, gold sputter–coated, and observed under SEM (JEOL JSM-6610LV, JEOL Inc, Peabody, MA, USA) at a magnification of 2000×. Each dentin disc was initially observed at low magnification, and then a representative area of treated dentin was chosen and recorded at the desired magnification.

Statistical Analysis

DP in percentage change from the baseline value was analyzed among the three periods in each paste using the Friedman test and Wilcoxon signed rank test. DP of the two crèmes were statistically compared at each period using the Mann-Whitney U-test. The significance level was set at 0.05.

RESULTS

Dentin Permeability

DP (%) changes from baseline values are presented in Figure 1. After application of CPP-ACP for five minutes, two weeks, and after acid challenge, the DPs were –0.45%, –64.99%, and –62.84%, respectively. After application of ACPP-ACP at the three

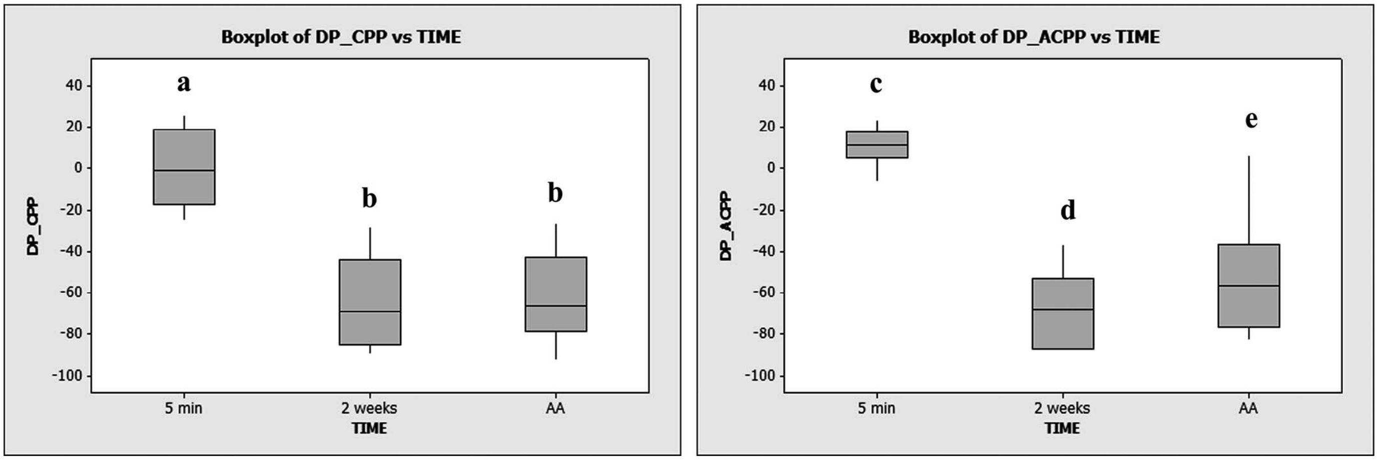

Figure 1. *Box plot representing percentage change of dentin permeability (DP) from baseline after application of CPP-ACP and ACPP-ACP at five minutes, two weeks, and after acid challenge (AA). The different labels indicate a significant difference among DP at the observation periods within a group. No significant difference was found between DP of CPP-ACP and ACPP-ACP at any time period.*

periods, the DPs were +10.91%, –68.39%, and –52.52%, respectively.

Comparing among periods, the DP of CPP-ACP was significantly different ($p<0.01$). DP at either two weeks or after acid challenge was significantly lower than that at five minutes ($p=0.018$). However, DP at two weeks and after acid challenge was not significantly different ($p\geq0.05$).

Comparing among the periods, DP of ACPP-ACP was significantly different ($p<0.01$). DP at either two weeks or after acid challenge was significantly lower than that at five minutes ($p=0.028$). In addition, DP at two weeks was significantly lower than that after acid challenge ($p=0.018$).

At five minutes, two weeks, or after acid challenge, DP of CPP-ACP application was not significantly different from that of ACPP-ACP ($p\geq0.05$).

Scanning Electron Micrographs

From the SEM micrographs, it was seen that the smear layer and plugs were completely removed from the dentin surfaces treated with 17% EDTA (control), with patent dentinal tubules (Figure 2A). After 5-minutes' application with CPP-ACP, fine-grained particles were partially observed on the dentin surface, with most tubular orifices remaining patent (Figure 2B). After the two-week application, the tubule orifices were partially blocked with deposits (Figure 2C). After the acid challenge with 6% citric acid, the dentinal tubules remained partially occluded, but to a lesser degree (Figure 2D).

Using ACPP-ACP for five minutes, 2-4-µm particles were partially observed on the dentin surfaces, with patent dentinal tubules (Figure 2E). After the two-week application with ACPP-ACP, the dentinal tubules were partially occluded with precipitates (Figure 2F). After acid challenge, the dentinal tubules remained partially blocked, but patent tubules were also observed in some areas (Figure 2G).

Diluted CPP-ACP–containing crèmes did not induce any precipitation at any of the observation stages, and all dentinal tubules remained patent (Figure 2H,I).

DISCUSSION

CPP-ACP–containing crèmes reduced DP and produced a partial blockage of dentinal tubules after two weeks' of daily application. The main precipitate from CPP-ACP is dicalcium phosphate dihydrate ($CaHPO_4 \bullet 2H_2O$).[13] However, the incomplete ability to decrease DP and occlude the dentinal tubules may be explained by a slow release of calcium and phosphate ions to form precipitates when CPP-ACP is neutral (pH 7).[13] Theoretically, dilution of CPP-ACP may enhance the release of ions and possibly improve the precipitation rate, but the release at neutral pH is much lower than under the acidic condition.[14] However, the effect on improving precipitation by diluting the CPP-ACP was not found in this study, wherein the dentinal tubules remained patent even after two weeks. Therefore, DP was not tested in the diluted CPP-ACP group in the current study.

CPP-ACP– containing crèmes should be consecutively applied for a period of time to establish and maintain optimal desensitizing effect.[12] For a five-

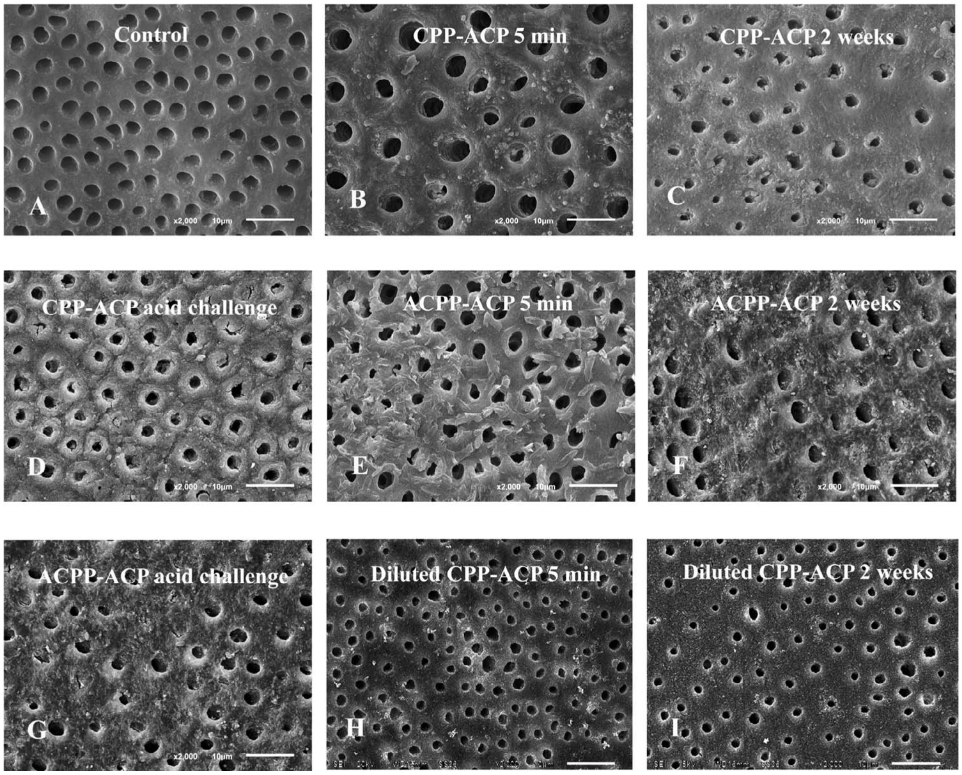

Figure 2. *Representative SEM micrographs (×2000 magnification) of dentin surfaces treated with CPP or ACPP at three different intervals. (A) Control; dentin treated with 17% EDTA presented patent dentinal tubules without smear layer or plugs. (B-D) CPP-ACP–containing crèmes at five minutes, two weeks, and after acid challenge with 6% citric acid. At five minutes, fine-grained particles were observed partially covering the dentin surface, with most tubular orifices remaining patent (B). At two weeks, the dentinal tubules were partially blocked with deposits (C). After acid challenge, the tubular orifices remained partially occluded, but to a lesser degree (D). (E-G) ACPP-ACP at five minutes, two weeks, and after acid challenge. At five minutes, particles, approximately 2-4 μm in size, were observed, with patent dentinal tubules (E). At two weeks, the dentinal tubules were partially occluded with precipitates (F). After acid challenge, the dentinal tubules remained partially occluded, but patent tubules were observed in some areas (G). (H-I) CPP-ACP–containing crèmes diluted with distilled water applied for five minutes or two weeks. All dentinal tubules remained patent without any precipitation that was similar to the observation after acid challenge.*

minute application, CPP-ACP was not effective in reducing DP or occluding the dentinal tubules; the majority of tubule orifices remained patent. This finding supports the clinical result by Kowalczyk and colleagues,[15] who reported that the therapeutic effect of CPP-ACP in relieving dentin hypersensitivity was insufficient when used over a short period. From our study, CPP-ACP should be used for at least two weeks to decrease DP and achieve a desensitizing effect. In addition, acidic foods or drinks should be avoided during the period because a lesser degree of tubular occlusion was observed after acid challenge.[16]

Theoretically, ACPP-ACP had a higher tendency to induce precipitation occluding dentinal tubules and decrease DP than did CPP-ACP. Microparticles were observed on the dentin surface after five-minute application. Immediate precipitation of ACPP-ACP is probably due to a rapid precipitation rate in the low pH environment as a calcium phosphate solution has been shown to have the highest precipitation rate at a pH of about 2.[13] ACPP-ACP was a diluted form of CPP-ACP that could increase the release of calcium and phosphate ions,[14] but the enhancing effect on precipitation was not found when CPP-ACP was diluted with distilled water. Thus, DP at five minutes was not reduced due to the limited effect of immediate precipitation. At two weeks, occlusion of the dentinal tubules when using ACPP-ACP tended to be more complete than with CPP-ACP. However, the results of DP were not different between the two pastes.

Apart from the precipitation, ACPP-ACP might interact with the dentin surface and dentinal tubules because of its slightly acidic nature. In evaluating the SEM image, however, we noted that the surface seemed to have become rougher and the tubules wider. This etching effect of ACPP-ACP should have increased DP. However, the effect of precipitates was likely to be stronger, so the DP was decreased after application of ACPP-ACP.

In this study, 6% citric acid was used to determine acid resistance of the precipitates. The SEM images showed that the precipitates occluding the dentinal tubules of the ACPP-ACP paste were less likely to be affected by the acid challenge than was CPP-ACP. This might be explained by the larger particles and the possibly faster rate of precipitate formation in the ACPP-ACP. After the two-week application, ACPP-ACP might induce a thicker precipitate layer, which is likely to resist the acidic conditions. However, DP after acid challenge was not different between CPP-ACP and ACPP-ACP.

In the ACPP-ACP group after acid challenge, the standard deviation of DP seemed to be higher than in the other groups. This may be explained by their capacity to resist the acid of the precipitation layer, which varied slightly among the specimens. As seen in the SEM image, the dentinal tubules in some areas were still protected while those in other areas were patent after acid challenge.

CONCLUSION

A two-week application of CPP-ACP–containing crèmes or ACPP-ACP decreased DP and induced dentinal tubule occlusion that resisted an acidic challenge. However, the efficiency in reducing DP of the CPP-ACP crèmes in a laboratory study may differ from the clinical situation with the presence of saliva, biofilm, or repeated acidic challenges. It is necessary to evaluate the clinical efficacy of the CPP-ACP–containing crèmes to confirm these laboratory results.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of Mahidol University. The protocol was approved by the Faculty of Dentistry and Faculty of Pharmacy Mahidol University Institution Regional Board (2015/032.1111).

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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