CALCIUM PHOSPHATE NANOPARTICLES REDUCE DENTIN HYPERSENSITIVITY: A RANDOMIZED, PLACEBO-CONTROLLED SPLIT-MOUTH STUDY

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ABSTRACT

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Introduction: Modern strategies for dental remineralization and prevention of dentin hypersensitivity are increasingly based on biomimetic materials. The aim of this study was to assess the clinical efficacy of a calcium-phosphate nanoparticles-based dentin desensitizer (DD, Teethmate Desensitizer, Kuraray Noritake, Japan).

Methodology: 25 patients, requesting treatment after reporting sensitivity of non-carious cervical lesions and dental abrasions were recruited. Inclusion criteria were: response score ≥6 on a 10 cm-long visual analog scale (VAS) for 1+ teeth in each of two quadrants. Exclusion criteria were: presence of systemic diseases, ongoing analgesic therapy, pregnancy, presence of carious or pulpal lesions, poorly contoured restorations, enamel cracks, active periodontitis and ongoing use of desensitizing agents. The response was determined to 2 s air blast. VAS scores were collected at t=0 (PRE), immediately after treatment with the DD (POST), after 1 week, and after 1, 3 and 6 months. Half of the sites in each patient (split-mouth) were randomly treated with a placebo, and scores collected until after 1 week. The DD was then applied to the placebo sites.

Results: Both DD and placebo significantly decreased VAS scores on POST confronted to PRE (p<0.0001), showing similar efficacy (35% and 28%, respectively). DD application further decreased scores after 1 week (63%) while placebo application did not show significant differences confronted to POST (p=0.09). DD scores maintained throughout the observational period the levels obtained after 1 week.

Conclusion: The tested DD effectively reduced dentin hypersensitivity during 6-month followup, after one single application. Biomimetic desensitizers may be an effective solution to dentin hypersensitivity.

Keywords: biomimetic material, dentin hypersensitivity, desensitization, calcium-phosphate nanoparticles.

1. Introduction

A short pain on exposed dentin, which can be elicited by thermal, evaporative, tactile, osmotic or chemical stimuli,¹ characterizes dentinal hypersensitivity. It is now widely acknowledged that dentin hypersensitivity derives from exposed dentin tubules, where stimuli excite pulp cells as proposed by Brannström's hydrodynamic theory.²,³ According to that theory, the diameter of the exposed tubules and their density are the factors that mainly influence hypersensitivity,⁴ as already highlighted by Yoshiyama et al.⁵ Finding a way to close the orifices of the tubules could

therefore decrease pain perception. Since this discovery, many therapeutic approaches to dentin hypersensitivity have been employed. 1.2.4.6 Less recent techniques were based on blocking the nociceptive conduction (mainly using potassium salts), on the superficial blocking of the orifices of the tubules with different compounds (varnishes, dentin bonding systems), or by inducing sclerosis of the tubules (SnF₂, SrCl*6H₂O, Al-, K-, Fe- oxalate, Fluorides, Na₂FPO₄). Lasers are also employed and have obtained positive results, but the exact mechanism is not fully understood, being likely due to either tubule occlusion or superficial glazing. 6

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A new possibility is provided by the introduction of biomimetic materials commonly used in dentistry. These materials can mimic a few properties of the tissue they are replacing, and in particular the use of nanotechnologies can help in obtaining synthesis and precipitation of hydroxyapatite at tooth level, opening possibilities for bioactive compounds and biomaterials to remineralize tooth structures, modulate biofilm formation, prevent caries occurrence and treat dentin hypersensitivity. Among several advantages that these remineralization techniques present, the possibility of closing exposed orifices of dentinal tubules using these materials is promising.

The aim of this study was to assess the clinical efficacy, expressed as pain reduction from hypersensitive tooth areas, of a dentin desensitizer (DD) based on calcium phosphate nanoparticles. The null hypotheses were that (i) no differences are shown between the tested DD and a placebo compound in pain reduction after one week of follow-up, and (ii) no significant differences in the efficacy of the test compounds are found among the evaluated time points.

2. Methods

2.1. Study design and materials

This study evaluated the efficacy of a calcium-phosphate nanoparticles-containing DD (test DD, Teethmate Desensitizer, Kuraray Noritake, Dental Inc. Okayama, Japan) on desensitization of non-carious cervical lesions and tooth abrasions. The test DD was compared with a placebo during the first 1 week of evaluation in a trial designed as a randomized, double-blind, placebo-controlled, split-mouth study. After that period, only the test DD was evaluated to assess its efficacy for up to 6 months. The composition of the materials used in this study are displayed in Table 1; the powder and liquid were transferred from the original packaging to glass bottles only identified by letters, masking their content to both patient and dental operator (double-blind trial).

The 'Guidelines for the design and conduct of clinical trials on dentin hypersensitivity' were adopted and partly followed during the design and execution of the study.¹¹

The study was conducted in agreement with the principles of the Declaration of Helsinki updated by the World Medical Association in 2013. ¹² Before obtaining written, informed consent, all patients

eligible for this trial received extensive verbal and written information regarding possible benefits and risks of the treatment. Patients were informed that one of the compounds tested is a placebo and that, if they felt the treatment did not reduce dentin hypersensitivity, they could feel free to exit the trial anytime and ask for alternative solutions.

2.2. Subject selection

Patients visiting 3 different private practice dental offices in Northern Italy and requesting treatment after reporting sensitivity were screened for eligibility. Non-carious cervical lesions or dental abrasions might be, or not, present at the hypersensitive sites. The inclusion criterion was a response score of ≥6 on a 10 cm long visual analog scale (VAS) that was numbered every centimeter from 0 to 10, for at least one tooth in each of two quadrants situated on the right-side and left-side of the mouth, so that a split-mouth model for test and placebo compounds could be applied.

Exclusion criteria were the presence of systemic diseases, ongoing temporary or permanent analgesic therapy for any reason, pregnancy, presence of carious or pulpal lesions, poorly contoured restorations, presence of enamel cracks on the hypersensitive teeth, active periodontitis and ongoing use of desensitizing agents of any kind. Patients were also excluded if the clinical situation requested other treatments on the screened sites than desensitizing alone.

The response was determined to a 2 s, cold air blast from a dental syringe directed perpendicularly to the tooth surface at approximately 5 mm distance. In the VAS scale, patients were told that 0 indicated a well-being condition with complete absence of any pain, while 10 was the worst pain they experienced or thought could ever exist. VAS scores collection was performed immediately after the air blast, patients being asked to point on the VAS scale to the nearest full centimeter number describing their pain perception.

A total of 25 patients were recruited, and each had the hypersensitive sites assigned to test or placebo treatment according to a randomization list. Neither the patient nor the dentist performing desensitizing treatments knew if the treated area belonged to the test or placebo compound, thus obtaining a double-blind model. The patients' demographics were as follows: 9 males (32-57 years old) and 16 females (25-60 years old).

Table 1. Composition of the tested materials. Placebo was obtained using reagents purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Material	Powder	Liquid
Teethmate Desensitizer	Tetracalcium phosphate, TTCP; dicalcium phosphate anhydrous, DCPA; sodium fluoride; glycerol; polyethylene glycol	Ultrapure water
Placebo	Glycerol monostearate; polyethylene glycol (PEG3350)	HPLC-grade water

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2.3. Application method

The targeted area was first cleaned with a cotton pellet. Then, the dentist mixed the powder with the liquid for 15 s to obtain a paste that was immediately applied on the treated areas with a microbrush by gently brushing for 2 min (Fig. 1 to 4). In the same visit, both test and placebo treatments were applied to each patient according to a randomization list. Patients were then asked to rinse their mouth, and within 15 min after the treatment the blinded dentist applied the same air blast stimuli under the same conditions to assess pain scores after treatments (POST). Patients were recalled for hypersensitivity assessment of the treated areas after 1 week. At that time point, after data collection, both dentist and patients were unblinded regarding the test or placebo treatment, and the test treatment was applied to the area that previously received the placebo compound. Patients were then recalled after 1, 3 and 6 months and at each recall the dentist assessed only tooth areas that were treated with test compound since the beginning of the trial.



Figure 1. Patient requesting desensitization treatment for vestibular areas of maxillary teeth. No presence of cervical lesions or abrasions could be identified as well as other lesions, yet upper right and upper left canines and premolars scored 7 on VAS after air blast test.



Figure 2. Application of the tested DD on right maxillary teeth (test site), same area depicted in Fig 1.

2.3. Statistical analysis

All statistical analyses were performed using statistical software (JMP 10.0, SAS Institute Inc, Cary, NC, USA). A preliminary check of the normality of distribution and homogeneity of variances was performed using Shapiro-Wilk's and Levène's tests (p<0.0001 and p=0.0014, respectively). Since data did not belong to continuous variable (0-10

ordinal VAS scores), were not normally distributed and homoscedasticity was not respected, non-parametrical ANOVA and non-parametric comparisons for each pair using Wilcoxon method (p<0.05) were used to highlight significant differences between groups.



Figure 3. Dental occlusal erosions bilaterally on palatal side of maxillary frontal and lateral teeth. Air blast test scored 8. Patient was referred to a gastroenterologist physician. He was later diagnosed with gastroesophageal reflux disease (GERD) and given treatment. Patient refused restorative therapies, requesting treatment of pain symptoms only.



Figure 4. Application of the test DD on the same area as in Fig 3.

3. Results

All 25 subjects completed the trial without requesting alternative desensitization treatments or dropping out from the trial. No adverse reactions were reported. The results of the study are displayed in Fig. 5. Both DD and placebo significantly decreased VAS scores comparing POST and PRE (p < 0.0001) measurements, thus showing similar efficacy (35% and 28%, respectively). DD application further decreased scores after 1 week (63% in comparison with PRE), while placebo application did not show significant differences when compared to POST (p=0.09). The scores from the areas treated with the DD maintained, throughout the observational period, the levels obtained after 1 week (maximum decrease in scores = 69% after 3 months). At 6 months, a small, non-significant increase in VAS scores was also observed.

4. Discussion

Dentin hypersensitivity is an increasing occurrence, and dental materials or procedures able to reduce the patient's sensitivity are increasingly needed. A variety of therapies are currently available, ^{2,4,6} but the most modern and biocompatible approach

seems to be the one aiming at reconstituting a barrier and closing the open orifices of the tubules by using biomimetic materials and techniques. 13,14,15,16

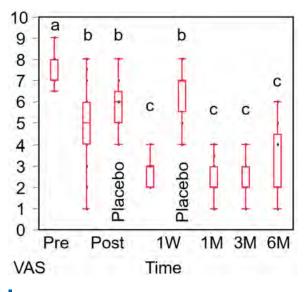


Figure 5. Box plot depicting the main findings of the study. The minimum and maximum values and the 25th, 50th, and 75th percentiles are shown in red. Different superscript letters indicate significant differences between groups (p<0.05) as assessed by Wilcoxon method. The test DD was compared with a placebo for up to 1 week, then its effect on reducing pain perception was monitored for 6 months.

In this study, we assessed the clinical efficacy, expressed as pain reduction from hypersensitive tooth areas, of a biomimetic, hydroxyapatite-forming dentin desensitizer (DD) based on calcium phosphate nanoparticles.

The first null hypothesis could not be fully rejected, since immediately after application there was no significant difference between DD under investigation and placebo, meaning that the reaction to the test DD immediately after application could not be due to its activity, but simply to a placebo effect. After one week, however, there was a highly significant difference between placebo and test DD. In fact, the test DD required 1 week to reach the significantly lowest scores of pain perception, and this level was maintained for up to 6 months. The second null hypothesis could, therefore, be rejected.

The tested DD is a biomimetic desensitizing compound based on the reaction between tetracalcium phosphate and dicalcium phosphate anhydrous in the presence of fluoride ions. Once water is added, the reaction produces hydroxyapatite and small parts of fluorapatite nanoparticles that precipitate as an amorphous layer on the tooth structures. Since nanocrystal deposition on enamel and dentin structures is driven by collagen backbone structures, one may speculate that the presence of these structures inside dentine tubules may help organize deposition of hydroxyapatite and fluorapatite. Thanatvarakorn et al. in 2013¹⁷ provided some data in support of this hypothesis. They showed that the tested DD developed an immediate reduction in dentin permeability and an effective integration of the calcium phosphate rich

layer with dentin surfaces, enhancing mineralization under oral conditions.¹⁷ The data obtained in this study could convey some indirect hints regarding the activity of the compound. It seems clear that the deposition of an amorphous layer of nanocrystals on the hypersensitive tooth surfaces does not lead to a reduction in pain perception greater than placebo effect. It is very likely, however, that hydroxyapatite and fluorapatite deposition inside dentinal tubules takes place at least over a one-week time period after application and is responsible for the significant reduction in pain perception when confronted to the placebo at 1 week or to the initial values. Our results confirm the assumptions of Zhou et al.¹⁰ thus providing a clinical confirmation of their in vitro results. In their study, the effectiveness of the same DD was evaluated in reducing dentin permeability and tubule orifice occlusion. It was found that the two parameters improved depending on the time until maximum values in permeability reduction and tubules occlusion were found after one week.¹⁰ These results are in good correlation with those of the present study, meaning that dentine permeability and occlusion of tubuli orifices (>50%) can be good indicators of reduction in pain perception.

In our study, it was found that the reduction in pain perception due to the test DD did not remain significantly different between the different time points starting from 1 week for up to 6 months. This means that the effects obtained by the tested DD (remineralization, reduction in dentin permeability and tubule occlusion)^{10,17} are long-lasting, however additional studies are needed to ascertain if the test DD may express its activity over extended observation times up to one or several years.

The study was performed under conditions as close as possible with those of similar studies 14,15 in order to evaluate possible differences yielded from geographical areas or pain perception. The tested DD compound was the same as the one used by Mehta et al. in 2014¹⁴ and similar to the one tested by the same research group one year after.¹⁵ The study design was the same as in Mehta et al., 2015, 15 except for the placebo follow-up that was stopped after 1 week in the present study. The statistical analysis was different in methodology, since in the present study no parametric analysis of data could be applied. One would speculate that, given that the material, the study design and the patient recruitment were very similar among these studies, similar results would be obtained. A first observation can be made comparing the initial pain scores to those after treatment and 1-week follow-up. The initial scores in the present study were higher than those from Mehta et al.,14,15 and reduction in pain perception after at least one week of treatment was higher than the results of Mehta et al., 15 but similar to the results the same Authors obtained one year earlier.14

Many different explanations for these results may be provided, including the fact that pain remains an extremely subjective perception being influenced by many confounding factors such as the socioeconomic-religious environment. This difference shows the importance of performing several studies with different populations from as many different

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environments as possible, particularly when testing the clinical behavior of therapies influencing a patient's pain perception. From this point of view, the setting itself where the investigations were carried out may be an additional confounding factor, since in the present study it was constituted by some private dental clinics while Mehta et al. evaluated patients seeking cure at a hospital Dental College. 14,15 Apart from the aforementioned factors, this distinction may have had an influence on the general health expectations patients had.

An additional difference between our study and that of Metha et al. 15 is that the latter evaluated Teethmate AP paste, produced by the same manufacturer as the desensitizer tested here. The main difference between the two products is that the paste is a waterfree calcium phosphate compound, while the test DD used in this study is a mixture of calcium phosphate powder with water, containing an accelerator that leads upon mixture and application to the reaction and precipitating hydroxyapatite nanocrystals.¹⁸ Comparing all findings, the pain reduction obtained by the paste desensitizer was slower and more moderate than that of the DD tested

According to Holland et al.,¹¹ assessments were made between contralateral teeth, and one side of the mouth served as a control for the other side (split-mouth model). A randomization list was used to assign each side to either test or control treatments. The comparisons were made between an active treatment (test DD) and a placebo that had aspect and composition identical to the active treatment, save for the active principles. In a previous study¹⁵comparisons were made between a similar DD and distilled water (placebo) for up to 6 months. In a pilot study we performed on 3 patients (data not shown), we saw that differences between placebo and test compounds were noticeable as of 1 week after treatment. It must be noted that all enrolled patients of this study reported discomfort levels in response to air blast ≥6 on VAS scale, and the tested treatment aimed to reduce pain perception. Contrary to the study design adopted by Mehta et al.,15 in the present study it was therefore decided not to prosecute observations on the placebo group for longer than the minimum amount of time necessary to assess a difference between placebo and test (1 week), since this would provide patients with further, unnecessary discomfort.

Another difference in the study design regarded the assessment of hypersensitivity, since in the present study, contrary to Mehta et al., 14,15 it was decided not to use the scrape test. The guidelines on conducting trials on dentin hypersensitivity suggested, as an example of response-based method, the use of cold stimuli, such as timed air blast, or tactile stimuli.11 In the study conducted by Mehta et al, as well as in many other studies, response was evaluated using both air blast and the tactile running of a dental explorer across the cervical area of the assigned teeth, in horizontal and vertical direction at a "relatively mild force". If one considers the microscopical and submicroscopical structure of enamel and dentine, however, it is clear that the tactile test is invasive and may produce damage to tooth structures at that level. Furthermore, tactile tests, if applied immediately after desensitization, may locally disturb the precipitated layer of nanocrystallites, hampering their further deposition and organization to occlude dentin tubule orifices. For this reason, any contact test was avoided in the present study, and further studies may be performed to assess the influence of tactile stimuli on the behavior of biomimetic hydroxyapatite-forming dentin desensitizers.

5. Conclusion

The results obtained in this study showed that the tested DD effectively reduced patient discomfort caused by dentin hypersensitivity during a 6-month follow-up, up to 69% after 3 months, after one single application. Biomimetic, hydroxyapatite-forming desensitizers may be an effective solution to dentin hypersensitivity.

Disclosure

No conflict of interest exists for any of the Authors of the paper.

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Questions

What is a biomimetic material?

- ☐a. A material that is only made of biological parts taken from natural tissues;
- □b. A material able to reproduce some features and properties of a natural tissue;
- ac. A material that reacts with the environment in a different way than a natural tissue does;
- □d. A material that does not react in any way with the environment or with the host.

Which one cannot be the mechanism of action of a dentin desensitizer?

- □a. It can block pain stimuli by blocking nervous transmission, for instance using potassium salts;
- □b. It can demineralize dentin surface, thus leaving more tubule orifices open to the oral environment;
- □c. It can cause sclerosis of the tubuli, thus reducing their lumen until the tubule is closed;
- □d. It can cause deposition of crystals inside tubuli, thus closing their orifices.

Which of these factors must be exclusion factors for subject recruitment when conducting a trial on dentin sensitivity?

- □a. Subjects already using a desensitizing toothpaste;
- □b. Subjects permanently using analgesic therapies;
- □c. Subjects younger than 30;
- ☐d. Subjects presenting carious lesions.

If a test treatment shows a positive effect confronted to the baseline, but the effect is nonsignificantly different from that of a placebo, what conclusions can be drawn?

- ☐a. The test compound is performing better than the placebo;
- □b. The test compound is not safely applicable to human subjects;
- □c. The test compound does not perform better than the placebo;
- □d. The placebo effect is not visible in the test.

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