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Promotion of enamel caries remineralization by an amelogenin-derived peptide in a rat model

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ABSTRACT

Objective: An amelogenin-derived peptide has been shown to promote remineralization of demineralized enamel in an *in vitro* model of initial caries induced by pH cycling. The present study examines whether the peptide exerts similar effects within the complex oral environment *in vivo*.
Design: Specific pathogen-free Sprague-Dawley rats (n = 36) were infected with *Streptococcus mutans*, given *ad libitum* access to Diet 2000 and drinking water supplemented with sucrose (10%, w/v), and then randomly divided into three groups treated with 25 μM peptide solution, 1 g/L NaF or deionized water. Molar teeth were swabbed twice daily with the respective solutions for 24 days. Then animals were killed, their jaws were removed and caries lesions were analyzed using the quantitative light-induced fluorescence-digital (QLF-D) technique to measure changes in mineral content. To verify QLF-D results, caries were scored for lesion depth and size using the Keyes method, and analyzed using polarized light microscopy (PLM).
Results: Mineral gain was significantly higher in teeth treated with peptide or NaF than in teeth treated with water ($p < 0.05$), based on the QLF-D results (ΔF and ΔQ). Incidence of smooth-surface and sulcal caries based on Keyes scores was similar in rats treated with peptide or NaF, and significantly lower in these groups than in rats treated with water ($p < 0.05$). Lesions on teeth treated with peptide or NaF were shallower, based on PLM. No significant differences were observed between molar enamel caries treated with peptide or NaF.
Conclusions: This amelogenin-derived peptide can promote remineralization in a rat caries model, indicating strong potential for clinical use.

1. Introduction

Dental caries is one of the most common chronic diseases in the world. It is a product of the interaction of many factors, including bacteria, fermentable carbohydrates and hard tooth tissue. When this interaction disturbs the balance of de- and re- mineralization of calcium and phosphate into and out of tooth enamel, dental caries can result (Ehrlich, Koutsoukos, Demadis, & Pokrovsky, 2009). Numerous studies have focused on preventing caries by promoting remineralization using such agents as fluoride. While fluoride has greatly reduced the prevalence of dental caries as one of the non-invasive agents most commonly used to manage non-cavitated caries lesions, it has also increased the prevalence of dental fluorosis (Pendrys, 2000; Wong et al., 2010).

Biomimetic remineralization has attracted increasing attention because of its potential to serve as an anti-caries agent (Chen, Yuan, Tang, Liang, & Li, 2015; George & Veis, 2008. Repeats of the sequence Asp-Ser-Ser (DSS) from dentin phosphoprotein, which is involved in mineralization of dentin extracellular matrix during tooth development, can bind tightly and specifically to hydroxyapatite (Yarbrough et al., 2010). There it attracts free ions from artificial saliva onto the surface of acid-eroded enamel, improving the surface properties (Chung, Li, & Hsu, 2012; Hsu, Chung, Yang, Shi, & Wu, 2011). Our group has shown that the peptide containing 8 repeats of DSS (8DSS) can promote remineralization of initial enamel caries lesions induced *in vitro* by pH cycling (Yang et al., 2014).

We have also shown that enamel matrix proteins (EMPs) can significantly inhibit demineralization of bovine enamel (Ran et al.,

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2014) and may promote remineralization of initial enamel carious lesions (Xiang et al., 2013). This is consistent with the known roles of EMPs in initiating enamel mineralization, supporting crystal growth at correct ultrastructural locations, protecting the growing mineral phase and arranging the crystals into discrete prism bundles (Ieong, Zhou, Li, Li, & Zhang, 2011; Moradian-Oldak, 2012). Our previous work was based on Emdogain (Straumann, Basel, Switzerland), a commercially available EMP mixture consisting nearly entirely of amelogenin (>95%) (Chung et al., 2012). Amelogenin-derived peptides that retain amino acids particularly important for crystal growth may also be effective for enamel biomimetic remineralization (Fan et al., 2011, 2012).

Focusing on the highly conserved QPX sequences in amelogenin, we designed an amelogenin-derived peptide containing five Gln-Pro-X repeats (QPY QPV QPH QPM QPQ) and a 7-residue hydrophilic segment (TKREEVD). We expected this peptide to serve as a biomimetic scaffold for nucleating hydroxyapatite and promoting mineralization, based on the predicted functions of Gln-Pro-X repeats (Lagunez-Otero, Diaz-Villasenor, & Renugopalakrishnan, 2002). *In vitro* studies of initial caries in bovine enamel showed that the peptide significantly promoted remineralization (Lv et al., 2015). This raised the question of whether the peptide would exert similar effects in the complex oral environment *in vivo*. Numerous *in vivo* factors are thought to influence remineralization in initial enamel erosion, including saliva composition, flow rate and pH (de Alencar et al., 2014). The presence of biofilms can also influence the outcomes of anti-caries treatments (Zhang et al., 2015).

The present study aimed to determine whether our novel amelogenin-derived peptide promotes remineralization in a rat model of enamel caries. This work is, to our knowledge, the first use of the quantitative light-induced fluorescence-digital (QLF-D) technique to quantify mineral change in an animal model of caries lesions.

2. Experimental

2.1. Amelogenin-derived peptide

The peptide sequence (QPY QPV QPH QPM QPQTKREEVD) was synthesized by GL Biochem (Shanghai, China) using standard Fmoc solid-phase chemistry on an Apex 396 multiple peptide synthesizer (AAPPTec, Louisville, KY). Peptides were purified to 95% purity using reverse-phase highperformance liquid chromatography.

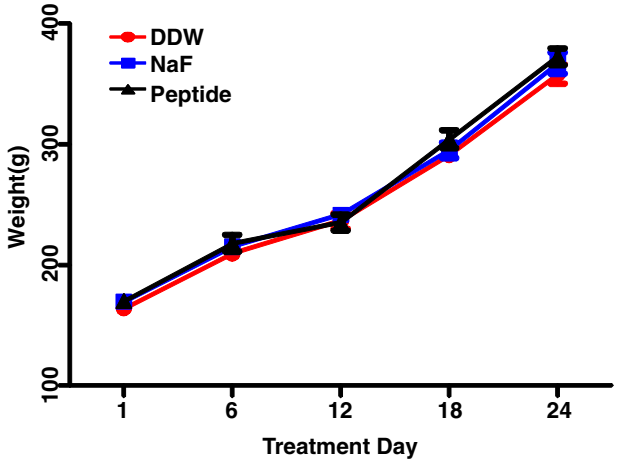

Fig. 1. Weight changes during treatment in Sprague-Dawley rats infected with *Streptococcus mutans* and subsequently treated on molars with distilled and deionized water (DDW), NaF or an amelogenin-derived peptide. Data shown are mean ± SD for 12 animals per treatment. Weight was similar for all groups throughout the treatment period.

2.2. Bacterial strain and inoculum

Streptococcus mutans strain ATCC27157 was provided by the State Key Laboratory of Oral Diseases of the Stomatology Department of West China Hospital, Sichuan University in Chengdu, China. *S. mutans* was subcultured from vial stocks onto *Mitis salivarius* agar (Sigma) at 37 °C in an atmosphere of 80% N_2 and 20% CO_2. Bacterial inoculum was prepared from 24-h cultures in brain heart infusion medium (Difco). Suspensions of 7×10^8 CFU/ml were obtained by adjusting cultures to the appropriate turbidity measured in an optical spectrophotometer (Spectronic 200, Thermo Fisher Scientific, India).

2.3. Rat caries model

Animal procedures in this study complied with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 8023, revised 1978). Specific pathogen-free, 12-day-old male Sprague-Dawley rats and their dams were obtained from the Experimental Animal Center of Sichuan University. Oral swabs from dams were streaked onto *Mitis salivarius* agar (Difco) and cultured anaerobically at 37 °C to confirm the absence of endogenous *S. mutans* infection. When pups were 20 days old, they were weaned and their weight was recorded. On the next day, animals were infected with *S. mutans* ATCC27157 using individual cotton swabs immersed in 1 mL of bacterial inoculum. This infection procedure was repeated on the following two days. When pups were 25 days old, infection was confirmed by taking oral swabs and streaking them onto *Mitis salivarius* agar. Over the next two weeks, animals were given *ad libitum* access to Diet 2000 (28% sucrose, 28% glucose) and drinking water supplemented with sucrose (10%, w/v).

2.4. Treatments

At the end of the 2-week period, animals were randomly allocated into three groups (n = 12 per group), who were housed in 12 cages with 3 animals each. From when animals were 40 days old until they were 63 days old, the groups were treated with 25 μM peptide solution, 1 g/L NaF solution or distilled and deionized water (DDW). Treatments were performed twice a day, at 08:00 and 20:00, by immersing individual cotton swabs in the appropriate solution held in centrifuge tubes and swabbing the molars. Each treatment lasted approximately 1 min. Food and water were withheld for 2 h afterwards.

All animals were weighed weekly and their physical state was recorded. They were killed by CO_2 asphyxiation at the age of 64 days.

2.5. Quantitative light-induced fluorescence-digital (QLF-D) technique

Jaws were hemisected, rinsed briefly in 10% formalin, placed in a labeled tube, and imaged under darkroom conditions. A QLF-D camera handpiece was attached to a vertical camera stand at a fixed distance of 5 cm above a black cardboard plate. Jaws were positioned with the occlusal side up and stabilized with wax to ensure measurement of fluorescence perpendicular to the occlusal plane (Alammari, Smith, de Josselin de Jong, & Higham, 2013). Jaws were allowed to air-dry for 5 sec, then a white-light digital image was taken of the tooth's occlusal surface. Next, a QLF-D image was obtained using a portable QLF-D device (C3 v1.25, Inspektor Research Systems, Amsterdam, The Netherlands) (Everett et al.,