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Effect of polyacrylic acid on dentin protease activities

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ABSTRACT

Objective. This study tested whether treatment of demineralized dentin with polyacrylic acid (PAA) has any activatory or inhibitory activity on dentin matrix metalloproteinases (MMP)s or cathepsin K (CAT-K).

Methods. Dentin beams (1 mm × 2 mm × 6 mm; $n = 10$) were completely demineralized with EDTA. After initial dry mass assessment, the beams were dipped into 37% phosphoric acid (PA), PA + 2% benzalkonium chloride (BAC), PA + 2% chlorhexidine digluconate (CHX), 10% PAA, PAA + BAC or PAA + CHX for 20 s. Demineralized beams without treatment served as control. All beams were incubated in simulated body fluid (SBF) for 1 week and the dry mass loss was evaluated. Aliquots of SBF were used to analyze solubilized telopeptide fragments using ICTP as indicator of MMP-mediated collagen degradation and CTX for CAT-K-mediated degradation. Additional demineralized beams ($n = 10$) were used to measure the influence of different chemical treatments on total MMP activity of EDTA-demineralized dentin using generic MMP assay. Data were analyzed by ANOVA ($\alpha = 0.05$).

Results. Dry mass loss ranged from 6% (PA) to 2% for (PA-BAC) or (PAA-BAC) ($p < 0.05$). ICTP release of PAA-treated group was significantly higher ($p < 0.05$) than the control, and not significantly different from the PA group ($p > 0.05$). PA + CHX or PAA + CHX and PAA + BAC showed significantly lower ICTP than PA or PAA groups ($p < 0.05$). CAT-K activity increased significantly after 10% PAA treatment compared to control ($p < 0.05$) or to PA postreatment.

Significance. Demineralized dentin treated with 10% polyacrylic acid activated CAT-K more than 37% phosphoric acid; 2% chlorhexidine digluconate seems to be a better inhibitor of MMPs and CAT-K than 2% benzalkonium chloride.

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1. Introduction

Although resin-enamel bonds are very stable over time [1], *in vivo* evidence indicates that resin–dentin bonds are prone to slow degradation in the oral environment [2–5]. The mechanism responsible for this degradation was first reported by Armstrong et al. [6], who published transmission electron microscopy images of normal *vs.* degraded hybrid layers in extracted human teeth that had been aged for four years *in vitro*. Those images showed the disappearance of collagen fibrils from hybrid layers. That same year, Pashley et al. [7] published results demonstrating that human dentin powder contained endogenous proteinases that could be inhibited by a variety of protease inhibitors, especially chlorhexidine, a potent antimicrobial agent known to inhibit matrix metalloproteinase (MMP)-2,-8 and -9 [8] and cysteine cathepsins [9].

More recently, cysteine cathepsins RNA was isolated from odontoblasts and pulp tissue [10] and both cathepsin B and cathepsin K (CAT-K) were identified in carious dentin suggesting the role of endogenous dentin proteases also in caries progression [11]. Therefore, an increasing amount of research is focused on how to inhibit or inactivate dentin proteases.

During the application of etch-and-rinse adhesives on dentin, phosphoric acid (PA) is used to remove the smear layer and to demineralize dentin to a depth of 5–8 μm [12]. This step enables infiltration of the dentin collagen matrix by adhesive resin monomers for micromechanical retention of resin composites. Unfortunately, both strong acids as well as mild acidic primers used at self-etch adhesives can activate the inactive proforms of endogenous dentin proteases [13–16].

Polyacrylic acid (PAA) etching is commonly employed in restorative dentistry to lightly etch dentin in preparation for placement of glass-ionomer cements. These cements contain aluminosilicate glass fillers that, when etched by PAA, release Al^{+++}, Ca^{++} and other ions that form a rigid, metallic salt bridge with polyacrylates [17]. Polyacrylic acids are polyanionic weak acids that may bind to dentin collagen matrices and endogenous proteases (*e.g.* MMPs and cathepsins). Although it is unlikely that a 10–15 s treatment of normal mineralized dentin will etch deep enough into mineralized normal dentin to activate the endogenous proteases of dentin, caries-affected dentin is already partially demineralized.

The authors previously reported that polyvinyl phosphonic acid, another linear polyanionic polymer, inhibits matrix-bound MMPs in dentin [18]. Hence, it was speculated that PAA may also have inhibitory effect on both MMP-mediated and CAT-K mediated degradation of demineralized dentin. Accordingly, the objective of the present study was to evaluate the potential of PAA as an inhibitor of endogenous protease activities in ethylenediamine tetra-acetic acid (EDTA)-demineralized human dentin. The null hypotheses tested were: (1) PAA has no effect on the total MMP activity in demineralized dentin collagen matrices, and (2) combining PAA with other known inhibitors such as chlorhexidine or benzalkonium chloride has no effect on the loss of dry mass or release of MMP-mediated carboxyterminal cross-linked telepeptide (ICTP) or CAT-K mediated cross-linked C-terminal telopeptide (CTX) of type I collagen from EDTA-demineralized dentin over time.

2. Materials and methods

2.1. Specimen preparation

Eighty unerupted molars were obtained from 18 to 21-year-old patients under a protocol approved by the Human Assurance Committee of Georgia Regents University. The teeth were stored frozen until use. After thawing, the enamel and superficial dentin of each tooth were removed using an Isomet saw (Buehler Ltd., Lake Bluff, IL, USA) under water cooling. Dentin beams with dimensions 6 mm × 2 mm × 1 mm were sectioned from the mid-coronal dentin (160 beams). All beams were completely demineralized in 0.5 M EDTA (pH 7.4) for 30 days at 4 °C with constant stirring. Three point flexure was used to confirm the absence of residual mineral. Mineralized beams have a modulus of elasticity of 16–19,000 MPa, while demineralized beams have a modulus of elasticity of 2–2.5 MPa [19]. Ten beams were assigned to each of 8 groups ($n = 10$).

2.2. Total MMP activity of demineralized dentin

Generic colorimetric MMP assay was used to determine if acid pretreatments could inhibit dentin-derived endogenous MMPs, using 10 EDTA-demineralized beams for each group. After demineralization, the beams were individually incubated in 300 μL of chromogenic thiopeptide substrate and assay buffer (Sensolyte Generic MMP assay; Anaspec, San Jose, CA, USA) in a 96-well plate for 60 min at 25 °C. After 60 min, the beams were removed from the wells; the 96-well plate was placed in a microplate reader (Synergy HT; BioTek Instruments, Winooski, VT, USA) to measure the baseline total MMP activity of each beam at 412 nm [20]. The beams were rinsed free of MMP assay substrate and then distributed to different groups such that the mean baseline activity of each group was not statistically significant. The beams were dipped in the respective acid solutions (Table 1), rinsed and incubated in fresh chromogenic substrate and assay buffer in the 96-well plate for 60 min at 25 °C. After 60 min of incubation, the activity was reassessed as described above. The total MMP activity was expressed as a percentage of the untreated baseline level to determine the percent inhibition or activation.

2.3. Loss of dry mass

After demineralization, a set of beams ($n = 10$/group) was transferred to individually labeled polypropylene tubes and placed in a desiccator containing anhydrous calcium sulfate (Drierite, W.A. Hammond Drierite Co., Xenio, OH, USA). With the cap off, each beam in separate tubes was desiccated to a constant mass within 72 h. The initial dry mass was measured to the nearest 0.001 mg using an analytical balance (XP6 Microbalance, Mettler Toledo, Hightstown, NJ, USA). The beams were distributed to the 8 experimental groups such that the mean initial dry mass of each group was similar in all groups. After dry mass measurement, the beams were completely rehydrated in deionized water to recover their original dimensions [19,21]. The rehydrated dentin beams were then immersed in the respective acid solution for 20 s (Table 1). After acid treatment, the beams were dropped in 50 mL of buffered