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Original Article

Effects of fluoride and epigallocatechin gallate on soft-drink-induced dental erosion of enamel and root dentin

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Background/Purpose: Fluoride and epigallocatechin gallate (EGCG) have been proven to prevent dental caries. The purpose of this study was to evaluate the effects of fluoride and EGCG on soft-drink-induced dental erosion *in vitro*.
Methods: Forty enamel and dentin specimens were prepared from extracted human teeth. The specimens were divided into 4 groups and treated separately with distilled water (as control), 0.5 M sodium fluoride (NF), 400 μM EGCG (EG), and a solution containing 0.5 M NaF and 400 μM EGCG (FG). Cyclic erosive treatment was performed according to the experimental procedures. The specimens were analyzed using laser scanning confocal microscopy, scanning electron microscopy, and a microhardness tester. The data were analyzed using ANOVA and Bonferroni's post hoc test. The significance level was set at 5%.
Results: The amount of substance loss was lower in the NF and EG groups than in the control group ($p < 0.05$). The erosion-caused substance loss was more pronounced in the dentin than in the enamel specimens. Surface microhardness loss was lower in the NF and EG groups than in the control group ($p < 0.05$). The diameter of the dentinal tubule was wider in the control group than in the NF and EG groups ($p < 0.05$). No combined effects were observed in the FG group.
Conclusion: Both fluoride and EGCG are effective in preventing soft-drink-induced erosion compared with the control group. Fluoride and EGCG may interfere with each other. The mechanisms of the anti-erosive effect need to be explored in the future.

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Introduction

Dental hard tissue, which is a highly mineralized structure, may be destroyed by contact with acidic substances. Common acid-related dental problems are caries and erosion, although their mechanisms differ. Dental erosion is gradually increasing in importance; it has been found to be a common condition in children and adolescents.[1] Dental erosion can have extrinsic or intrinsic causes. The primary extrinsic source of dental erosion is acidic food.[2] Diet, particularly the consumption of soft drinks, is the main etiological factor.[1] The amount of soft-drink consumption has increased dramatically since 1988. Sales of soft drinks increase by 56% between 1990 and 2000, and increase by 2–3% per year therefore.[3] In fact, there are many acidic soft drinks. Erosive potential of soft drinks may come from several properties, such as the type of acid contained, the pH value, the titratable acidity, etc. In a previous study, more tooth structure loss was caused by coke compared with other commercial soft drink products.[4]

Dental caries is a bacteria-associated disease. Progression of dental caries may induce serious clinical problems. Much effort has been focused on prevention of dental caries and fluoride has been proven to be effective for dental caries prevention. Calcium-fluoride-like minerals are deposited on the tooth surface when fluoride compounds are applied. During acidic challenge, fluoride is released and inhibits demineralization. When the pH is elevated, fluoride in the solution becomes supersaturated with respect to tooth mineral and subsequently speeds up the process of remineralization. Fluoride can also provide antimicrobial action, particularly against cariogenic bacteria, thus proving its effectiveness in the microbiological aspect of caries prevention.[5]

Epigallocatechin gallate (EGCG), also known as epigallocatechin-3-gallate, is a type of catechin and exhibits broad biological and pharmacological activities.[6] In addition, it offers high anti-caries efficacy, a property derived primarily from its antibacterial activity and the mechanism is related to its chemical structure. Ikigai et al. concluded that high EGCG concentrations irreversibly damage the bacterial cytoplasmic membrane.[7] In their study, phosphatidylcholine liposomes leaked fluorescent dye following exposure to the compound. However, the introduction of charged lipids into the liposomal bilayer, more appropriately reflecting the composition of the cytoplasmic membranes of gram-positive and gram-negative bacteria, reduced the extent of this damage. It has been claimed that cytoplasmic membrane damage results from the generation of hydrogen peroxide by EGCG within the phospholipid bilayer.[8] Several researchers have reported that catechins inhibit *Streptococcus sobrinus* and *Streptococcus mutans*.[9]

Erosion may cause irreversible tooth structure loss, and even induce dental hypersensitivity while the dentin is involved. About 15% of the populations suffer from erosion-induced dentin hypersensitivity.[10] Erosion of enamel is initially shown as demineralization at the surface, then proceeding with softening and substance loss. In dentin, mineral loss is initially at the border between peritubular and intertubular dentin. Subsequently, peritubular dentin is lost and tubule orifice is enlarged. It may increase the permeability of dentin tubule and contribute to hypersensitivity. For detection of dimensional changes of the dentinal tubules, there are many studies using SEM images to measure them directly or through software aids. Confocal microscope is a kind of optical microscope. Specimens can be examined directly without preparation. True 3D images can be obtained by confocal microscopy. Recently, the resolution of confocal microscope is approaching to the level of SEM. Few studies report on observation of dentin using confocal microscope.

Many researchers are focusing on the prevention of dental erosion induced by soft drinks. Although reduction of soft-drink consumption has been suggested, the addition of components to soft drinks can also efficiently reduce erosion; supplementing soft drinks with green tea extract can reduce the erosive potential against dentin.[11] Nevertheless, the altered taste is another concern. Enhancement of tooth property may be an alternative method for preventing erosion, such as improving acid resistance and reducing the demineralization effect. However, the conditions of the oral environment are highly complex. Caries and erosion may present simultaneously. The purpose of this study was to evaluate the effects of fluoride and EGCG on soft drink-induced dental erosion to determine whether it was possible to protect teeth from erosion by using materials with anti-caries effects.

Materials and methods

Preparation of tooth specimens

Forty enamel and dentine specimens were prepared from human teeth (third molars and premolars) which had been extracted for medical reasons. This study was approved by the Institutional Review Committee of National Taiwan University Hospital, Taipei, Taiwan (approval number: 201208032RIC). The procedure for preparation of specimens is described in brief. The crown and root portions of each tooth were separated by cutting at the cement–enamel junction, using an ISOMET Low Speed Saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA). Enamel samples were cut from the buccal/lingual surfaces of the crowns. Dentine samples were cut from the outer surface of the roots. Each tooth sample was embedded in cold cure acrylic resin as the mounting mode (size: 15 mm × 15 mm × 5 mm). The samples were polished sequentially, using silicon carbide paper of 800, 1200, 2500, and 4000 grit on a rotating polishing machine under constant water irrigation. The removal depth of specimens was no more than 500 μm. This resulted in an exposed area of enamel approximately 3 mm × 3 mm in size and dentine approximately 3 mm × 2 mm in size. Specimens were cleaned through ultrasonication in distilled water for 3 min after polishing. Half of each exposed specimen was covered with polyvinyl adhesive tape (Scotch 810; 3M, St. Paul, MN, USA) for reference. Tooth specimens were randomly divided into 4 groups, according to the following treatments: Group A: distilled water (DW) as control; Group B: 0.5 M NaF (NF, Sigma–Aldrich Co. LLC. St. Louis, MO, USA) solution; Group C: 400 μM EGCG (EG, Sigma–Aldrich Co. LLC. St. Louis, MO, USA); and Group D: NaF (0.5 M) + EGCG (400 μM) (FG).

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