Epigallocatechin-3-gallate Blocks Triethylene Glycol Dimethacrylate–induced Cyclooxygenase-2 Expression by Suppressing Extracellular Signal-regulated Kinase in Human Dental Pulp and Embryonic Palatal Mesenchymal Cells

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Abstract

Introduction: Methacrylate resin–based materials could release components into adjacent environment even after polymerization. The major components leached include triethylene glycol dimethacrylate (TEGDMA). TEGDMA has been shown to induce the expression of cyclooxygenase-2 (COX-2). However, the mechanisms are not completely understood. The aims of this study were to investigate the molecular mechanism underlying TEGDMA-induced COX-2 in 2 oral cell types, the primary culture of human dental pulp (HDP) cells and the human embryonic palatal mesenchymal (HEPM) pre-osteoblasts, and to propose potential strategy to prevent or ameliorate the TEGDMA-induced inflammation in oral tissues. **Methods:** TEGDMA-induced COX-2 expression and its signaling pathways were assessed by Western blot analyses in HDP and HEPM cells. The inhibition of TEGDMA-induced COX-2 protein expression using various dietary phytochemicals was investigated. **Results:** COX-2 protein expression was increased after exposure to TEGDMA at concentrations as low as 5 μmol/L. TEGDMA-induced COX-2 expression was associated with reaction oxygen species, the extracellular signal-regulated kinase 1/2, and the p38 mitogen-activated protein kinase signaling pathways in HDP and HEPM cells. The activation of p38 mitogen-activated protein kinase was directly associated with reactive oxygen species. Epigallocatechin-3-gallate suppressed TEGDMA-induced COX-2 expression by inhibiting phosphorylation of extracellular signal-regulated kinase 1/2. **Conclusions:** Cells exposed to low concentrations of TEGDMA may induce inflammatory responses of the adjacent tissues, and this should be taken into consideration during common dental practice. Green tea, which has a long history of safe beverage consumption, may be a useful agent for the prevention or treatment of TEGDMA-induced inflammation in oral tissues. *(J Endod 2013;39:1407–1412)*

Key Words

Cyclooxygenase-2, epigallocatechin-3-gallate, human embryonic palatal mesenchymal, pulp, triethylene glycol dimethacrylate

In recent decades, methacrylate resin–based materials have gained popularity over mercury-containing amalgam because of the high esthetic demands for dental restorative materials. In addition to the common use of these materials as direct dental filling materials, methacrylic compounds are also extensively incorporated in dental cements, dentin adhesives, and luting agents for crowns, inlays, and orthodontic brackets and in endodontic products, conventional bone cements, and scaffolds for tissue regeneration (1, 2). With the increased use of methacrylate resin–based materials, a major concern exists regarding their toxicity and adverse effects.

Methacrylate resin–based materials could release components into the adjacent environment even after polymerization (3). The major components leached from resin include triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate, bisphenol A–glycidyl methacrylate (BisGMA), urethane dimethacrylate, and other additives (3). Among these, TEGDMA is the main component released (3). TEGDMA can easily penetrate membranes and react with intracellular molecules (4). Leaching TEGDMA not only acts on adjacent tissues but also may distribute systemically to cause adverse effects. The concentrations of TEGDMA leaching from dentinal adhesives could reach pulp up to 4 mmol/L after diffusion through dentin in deep cavities (5). The unpolymerized TEGDMA in the pulp may then diffuse into the bloodstream (3). *In vitro* studies have shown that TEGDMA is highly cytotoxic and moderately genotoxic. The toxicity of TEGDMA is associated with glutathione depletion and excessive reaction oxygen species (ROS) production (6). In addition to its toxic effects, TEGDMA may reduce the differentiation and mineralization process of dental pulp cells and osteoblastic cells at sublethal doses (7).

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Methacrylate resin–based dental adhesives and root canal sealers are associated with pulpal and periapical inflammation (8, 9). TEGDMA has been shown to induce cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), and prostaglandin F2alpha production in human dental pulp cells (10). PGE2 and prostaglandin F2alpha are transformed from arachidonic acid by COX-2 (11) and are involved in inflamed and painful dental pulp (12). Therefore, COX-2 may play a role in the pathogenesis of pulpitis. However, the exact mechanisms of TEGDMA-induced COX-2 protein expression are not completely understood. A full understanding of the mechanism underlying TEGDMA-induced COX-2 may help to design a more effective strategy to prevent or ameliorate TEGDMA-induced inflammation in oral tissues. Therefore, we examined the possible signal transduction pathways involved in the TEGDMA-induced COX-2 protein expression in 2 oral cell types: the primary culture of human dental pulp (HDP) cells and the human embryonic palatal mesenchymal (HEPM) preosteoblasts.

Although selective COX-2 inhibitors are promising in managing endodontic pain and inhibiting periradicular inflammatory bone resorption (13), evidence suggests that COX-2 inhibitors are associated with serious cardiovascular adverse effects including heart attacks and death (14). Other strategies may be needed for the prevention or treatment of tissue inflammation caused by TEGDMA. Various dietary phytochemicals have been shown to suppress COX-2 expression. Therefore, we investigated whether curcumin, epigallocatechin-3-gallate (EGCG), lovastatin, and phenylethyl isothiocyanate (PEITC) had a protective effect on TEGDMA-induced COX-2 expression in HDP and HEPM cells.

Materials and Methods

Materials

TEGDMA, EGCG, curcumin, PEITC, dimethyl sulfoxide, and N-acetyl-L-cysteine (NAC) were obtained from Sigma-Aldrich (St Louis, MO). Lovastatin was purchased from Standard Chem & Pharm Co (Tainan, Taiwan). The specific inhibitor of extracellular signal-regulated kinase (ERK) inhibitor PD98059, c-Jun NH_2-terminal kinase inhibitor SP600125, p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580, nuclear factor κB (NF-κB) inhibitor Bay 11-7082, and phosphoinositide 3-kinase inhibitor LY294002 were purchased from Calbiochem (San Diego, CA). Antibodies for ERK1/2, phosphorylated ERK1/2, p38 MAPK, and phosphorylated p38 MAPK were from Cell Signaling Technology (Beverly, MA). Antibodies against COX-2 and beta-actin were from Santa Cruz Biotechnology (Santa Cruz, CA). All tissue culture biologicals were from Invitrogen (Carlsbad, CA).

Cell Culture

The protocol of this study has been reviewed and approved by the Ethic Committee of National Taiwan University Hospital. HDP fibroblasts were obtained from permanent non-carious premolars extracted from 5 patients for orthodontic reasons. Informed consents were obtained before extraction of the premolars. Five HDP cultures were established using an explant technique as previously described (15). Cells between 4 to 10 passages were plated on 60-mm Petri dishes at a density of 2×10^5 cells and subjected to various treatments. HEPM cells were obtained from American type culture collection (Livingstone, MT). The cells were serum starved for 18 hours before treatment. To identify the signal transduction pathways involved in TEGDMA-induced COX-2 expression, HDP and HEPM cells were pretreated with 4 mmol/L NAC for 2 hours, 20 μmol/L LY294002, 10 μmol/L SB203580, 10 μmol/L SP600125, 10 μmol/L PD98059, or 5 μmol/L Bay 11-7082 for 1 hour and then treated with 15 μmol/L TEGDMA for 9 hours. In all experiments, 0.05% dimethyl sulfoxide treatment was used as the control.

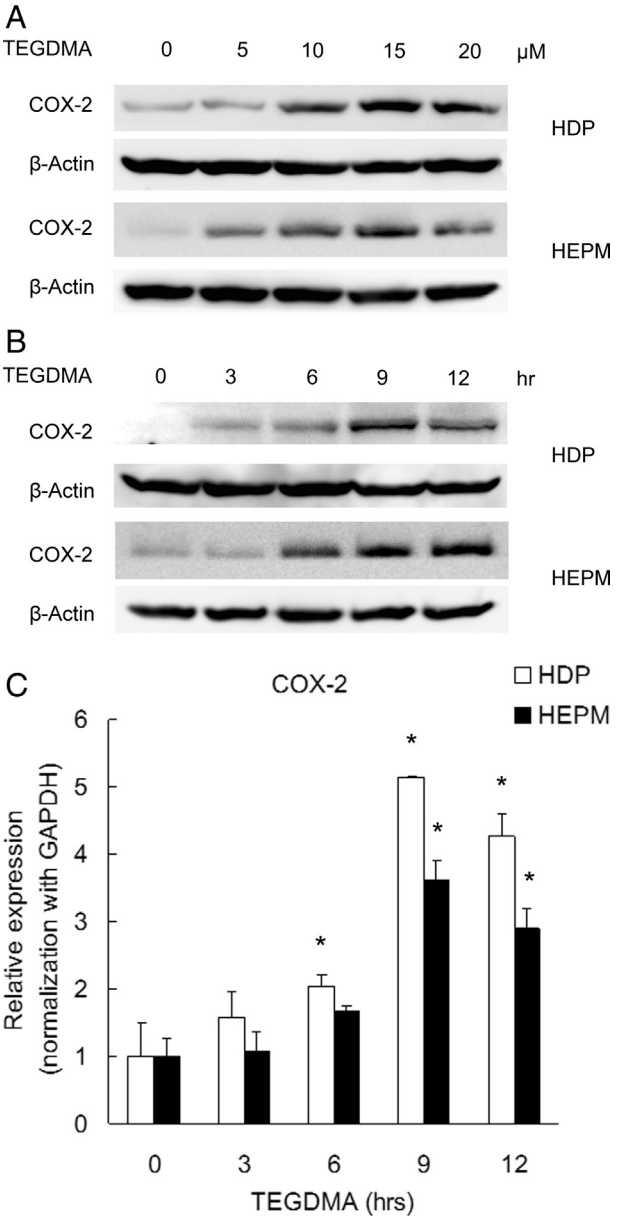

Figure 1. TEGDMA-stimulated COX-2 expression in primary culture of HDP cells and HEPM preosteoblasts. (*A*) Cells were treated with various concentrations (0–20 μmol/L) of TEGDMA for 9 hours. COX-2 protein levels were measured by Western blot analysis. (*B*) Kinetics of COX-2 induction using 15 μmol/L TEGDMA in HDP and HEPM cells. (*C*) Cells were treated the same as in *B*, and COX-2 mRNA levels in HDP and HEPM cells were measured using TaqMan Gene Expression Assays as described in the Materials and Methods section. The bars represent means $\pm$ standard error of the mean (n = 4, *P < .05 compared with the control).

Western Blot Analysis

Western blot analysis was performed as previously described (15). In brief, cells were lysed in lysis buffer, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred to PVDF membranes. The membranes were then incubated with antibodies against COX-2, ERK1/2, phosphorylated ERK1/2, p38 MAPK, phosphorylated p38 MAPK, or beta-actin followed by incubation with a horseradish peroxidase–conjugated secondary antibody