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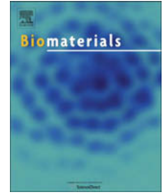

The effect of BisGMA on cyclooxygenase-2 expression, PGE_2 production and cytotoxicity via reactive oxygen species- and MEK/ERK-dependent and -independent pathways

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A B S T R A C T

After operative restoration, some monomers released from dentin bonding agents or composite resin may induce tissue inflammation and affect the vitality of dental pulp. Whether BisGMA, a major monomer of composite resin, may induce prostaglandin release and cytotoxicity to pulp cells and their mechanisms awaits investigation. We found that BisGMA induced cytotoxicity to human dental pulp cells at concentrations higher than 0.075 mM as analyzed by 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. BisGMA (0.1 mM) also stimulated ERK phosphorylation, PGE_2 production, COX-2 mRNA and protein expression as well as ROS production (as indicated by an increase in cellular DCF fluorescence) in dental pulp cells. Catalase (500 and 1000 U/ml) and U0126 (10 and 20 μM, a MEK inhibitor) effectively prevented the BisGMA-induced ERK activation, PGE_2 production and COX-2 expression. Moreover, catalase can protect the pulp cells from BisGMA cytotoxicity, whereas aspirin and U0126 lacked of this protective activity. These results suggest that BisGMA released from composite resin may potentially affect the vitality of dental pulp and induce pulpal inflammation via stimulation of ROS production, MEK/ERK1/2 activation and subsequent COX-2 gene expression and PGE_2 production. Cytotoxicity of BisGMA to dental pulp cells is related to ROS production, but not directly mediated by MEK activation and PGE_2 production.

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1. Introduction

Resin modified glass ionomers and composite resins have been widely used for restoration of caries cavity or fractured tooth structure with or without dentin bonding agents for a long time [1]. However, considerable amounts of components may release from adhesive materials and composite resin even after polymerization. The major components leached from resin-based materials and adhesives included triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), bisphenol-glycidylmethacrylate (BisGMA), urethane dimethacrylate (UDMA) and other additives [2,3]. Different amounts of these monomers are found to be eluted from light-cured dental resins and resin composites into water, methanol, and 75% ethanol/water solution as analyzed by gas chromatography or/and high performance liquid chromatography [4–6]. They pose potential adverse systemic effects or damage to adjacent dental pulp and other oral tissues [3].

Restoration of deep caries cavity in human teeth with dentin bonding agents may induce evident cytotoxicity, pulpal inflammatory responses, delayed pulpal healing with failure of dentin bridge formation [7,8]. The levels of many inflammatory mediators such as prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, Interleukin-8 (IL-8) etc. are elevated during pulpal inflammation [9–11]. Dentin adhesives and their monomer contents have been shown to stimulate the expression of inflammatory mediators in oral epithelial cells and dental pulp cells

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[12,13]. These results suggest that components in resin adhesives may potentially induce pulpal inflammatory responses and affect biological activity of pulp cells via activation of receptors such as prostaglandin EP and FP and downstream signaling [14,15].

Previous studies have suggested that cytotoxicity of dentin bonding agents and resin monomers is associated with glutathione (GSH) depletion and excessive reaction oxygen species (ROS) production [16–18]. ROS has been shown to induce oxidative DNA damage and trigger various signal transduction pathways including Ataxia-telangiectasia mutated (ATM), nuclear factor-kappa B (NF-kB) and mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK) and p38 [19–21]. Activation of MAPKs may potentially affect chemical cytotoxicity and mediate the changes in gene expression of inflammatory mediators such as IL-6 and cyclooxygenase-2 (COX-2) in different kind of cells [19,22–24]. N-acetyl-L-cysteine (NAC) as a precursor of GSH and direct ROS scavenger has been shown to regulate apoptosis, angiogenesis, and cell growth and inhibits inflammatory response [25]. Likewise, NAC may prevent the TEGDMA and HEMA-induced genotoxicity and cell cycle arrest in Chinese hamster V79 cells [26]. In this study, it is interesting to know whether BisGMA, as a major resin monomer in dentin bonding agents and composite resin, may induce reactive oxygen species (ROS) production, thereby stimulate MEK/ERK1/2 signaling transduction pathway, leading to cytotoxicity, COX-2 gene expression and PGE_2 production.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin were from Gibco (Life Technologies, Grand Island, NY). Ethidium bromide, agarose, and kits for reverse transcription (RT) and polymerase chain reaction (PCR) were purchased from HT Inc., UK. Total RNA isolation kits were from Qiagen (Qiagen Company, Taiwan). Specific primer sets for COX-2 and β-actin [14,22] were synthesized by Genemed Biotechnologies, Inc. (San Francisco, CA, USA). Random primers for reverse transcription were from Invitrogen Inc. (California, USA). Mouse antihuman-GAPDH (sc-32233), and p-ERK (sc-7383) antibodies and goat antihuman COX-2 antibody (sc-1745) were from Santa Crutz Biotechnology, INC. (California, USA). Protein assay kits were obtained from Bio-Rad (Bio-Rad Labs, Hercules, CA, USA). PGE_2 ELISA kits were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Catalase, 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), NAC, BisGMA and U0126 were from Sigma/Aldrich (Sigma Chemical Company, St. Louis, MO., USA). BisGMA was dissolved in dimethylsulfoxide (DMSO) and diluted sequentially to get different concentrations of stock solution before addition into culture medium. The final amounts of DMSO in the culture medium were same (0.2–0.3%, v/v) in different wells.

2.2. Culture of human dental pulp cells

Primary human dental pulp cells were cultured and characterized as described previously [14,15,17]. After proper informed consent, human premolars were freshly extracted during orthodontic treatment and split by a hammer. Pulp tissues were obtained, minced into small pieces and cultured in DMEM containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air/5% CO_2. Cultured dental pulp cells in passage numbers from three to eight and from several donors were used for these studies with similar results. No marked change in morphology and decline in proliferation of pulp cells were found within passage 8.

2.3. Cytotoxicity and morphological changes of dental pulp cells after exposure to BisGMA

Briefly, 4×10^5 pulp cells were inoculated into 6-well culture plates. After 24-h, medium was changed and then various concentrations of BisGMA or DMSO (control) were added. Cells were further incubated for 26-h and morphological changes were taken by a camera under phase contrast microscope. Medium was collected for further measurement of PGE_2. Then fresh medium with MTT (final 0.5 mg/ml) was added for 3-h. Medium was then decanted and the insoluble formazan was dissolved in DMSO and read against blank (DMSO) by a Dynatech Microwell plate reader (Dynatech Labs. Inc., Chantilly, VA, USA) at a wavelength of 540 nm [17,22]. In some experiments, cells were pretreated with aspirin (100 and 200 μM), catalase (500 and 1000 U/ml) or U0126 (10 and 20 μM) for 30 min prior to the addition of BisGMA, and then co-incubated for 26-h before MTT assay.

2.4. Effect of BisGMA on PGE_2 production in dental pulp cells and its modulation by aspirin, catalase, and U0126

Pulp cells were treated as above and culture medium was collected for measurement of PGE_2 concentration by the PGE_2 ELISA kits according to manufacture's instruction. In some experiments, cells were pretreated with aspirin (100 and 200 μM), catalase (500 and 1000 U/ml) or U0126 (10 and 20 μM) for 30 min prior to the addition of BisGMA, and then co-incubated for 26-h.

2.5. Effect of BisGMA on cyclooxygenase-2 (COX-2) mRNA expression in dental pulp cells and its modulation by catalase and NAC

Dental pulp cells (3×10^6 cells) were inoculated into 10-cm culture dishes for overnight and then exposed to various concentrations of BisGMA or solvent (as control) for 26-h. Thereafter, total RNA was isolated by Qiagen RNA isolation kits as described previously [14,22]. In some experiments, cells were pretreated with catalase (1000 U/ml) or NAC (5 mM) for 30 min prior to the addition of BisGMA and co-cultured for 26-h.

2.5.1. Semi-quantitative reverse-transcriptase and polymerase chain reaction (RT-PCR)

Brief, 3 μg of denatured total RNA was reverse transcribed in a total volume of 44.5 μl reaction mixture containing 4 μl of random primer (500 μg/ml), 8 μl of dNTP (2.5 mM), 4.5 μl of 10× RT buffer, 1 μl of RNase inhibitor (40 U/μl) and 0.5 μl of RT (21 U/μl) at 42 °C for 90 min. Four microliters of the RNA/DNA hybrid were then used for PCR amplification in a reaction volume of 50 μl containing 5 μl of 10× Super TAQ buffer, 4 μl of dNTP (2.5 mM), 1 μl of each specific primer, and 0.2 μl of Super TAQ enzyme (2 U/μl). The reaction mixture was initially heated to 94 °C for 5 min in the first cycle. The reaction was amplified for 15–35 cycles of 94 °C for 1 min, 55 °C for 1 min and then 72 °C for 2 min with a thermal cycler (Perkin Elmer 4800, PE Applied Biosystems, Foster city, CA, USA). Finally, the reaction was set at 72 °C for 10 min. The primer pairs used in this study were: β-actin (BAC) (control primers), 5′-AAGA-GAGGCATCCTCACCCT-3′ and 5′-AAGTTGTGGGGTCGGTACAT-3′ (218 bp). COX-2 is 5′-TTCAAATGAGATTGTGGGAAAATTGCT-3′ and 5′-AGATCATCTCTGCCTGAGTATCTT-3′ [14,22]. The amplified PCR product is 305 base pairs (bp) for COX-2. The PCR amplified products were loaded for 1.8% agarose gel electrophoresis in 1× of TBE buffer. After electrophoresis, the DNA products amplified by different PCR cycles were stained with ethidium bromide and the DNA bands photographed by using the Alpha-Imager 2000 (Alpha Innotech, San Leandro, CA, USA). The DNA bands which showed linear in relation to the PCR cycles were used for data presentation.

2.6. Effect of BisGMA on cyclooxygenase-2 (COX-2) protein expression and ERK1/2 phosphorylation in dental pulp cells

2.6.1. Western blotting

Cells were exposed to various concentrations of BisGMA for defined periods as described in figure legend. Cell lysates were prepared as described previously using freshly prepared lysis buffer (10 mM Tris–HCl, pH 7; 140 mM sodium chloride; 3 mM magnesium chloride; 0.5% NP-40; 2 mM phenylmethylsulfonyl fluoride; 1% aprotinin; and 5 mM dithiothreitol) [14,22]. The protein concentration of the cell lysates was measured by Bio-Rad protein assay kits. Equal amounts of protein (50 μg/lane) were separated by 12% SDS-polyacrylamide gel electrophoresis (Scie-Plas, UK) and transferred to PVDF membrane by electroblotting. The membrane was blocked for 30 min at room temperature in a blocking reagent (20 mM Tris, pH 7.4; 125 mM NaCl; 0.2% Tween-20; 5% nonfat dry milk; and 0.1% sodium azide) and then incubated for 2-h with mouse antihuman phospho-ERK1/2 and GAPDH antibodies and goat antihuman COX-2 antibody (1:500). Membranes were washed three times with TBST (10 mM Tris, pH 7.5; 100 mM NaCl, 0.1% Tween-20) for 10 min each, and then incubated with HRP-labeled goat anti-mouse secondary antibody for 1 h. The membrane was then washed 4 times with TBST. Finally the immunoreactive bands were developed by Enhanced Chemiluminescence (ECL) reagent and visualized on Fuji X-ray film.

2.7. Effect of BisGMA on reactive oxygen species production in dental pulp cells

Briefly, 4×10^5 pulp cells were seeded into 6-well culture plates. After 24-h, fresh medium with various concentrations of BisGMA or DMSO (control) were changed and further incubated for 3-h or 26-h. Cells were then stained with 10 μM of DCFH-DA for 30 min at 37 °C incubator, detached by trypsin/EDTA, washed with PBS and immediately subjected for flow cytometric analysis as described before [17,27]. Mean DCF fluorescence values were normalized to about 100 (control) and the DCF fluorescence of BisGMA-exposure groups relative to control were determined and used for data presentation.

2.8. Statistical analysis

Three or more separate experiments were performed. Raw data from separate experiments were combined. Results of MTT data were expressed as percentage of control (as 100%). PGE_2 levels in the culture medium were shown as pg/ml. All quantitative data were expressed as Mean ± SE and analyzed by One-way analysis of

variance (ANOVA) followed by post-hoc Bonferroni test. A p value < 0.05 was considered to have a significant difference between groups.

3. Results

3.1. Cytotoxicity of BisGMA to dental pulp cells

Exposure of dental pulp cells to BisGMA at concentrations of 0.075 and 0.1 mM markedly affected the viable cell number as analyzed by MTT assay with 40% and 66% of inhibition, respectively (Fig. 1). An elevation of BisGMA concentration to 0.25 mM led to almost total death of pulp cells.

3.2. BisGMA induced morphologic changes of pulp cells

As shown in Fig. 2A, untreated dental pulp cells showed spindle-shaped in appearance and extended cellular processes. After exposure to 0.075 mM BisGMA, retraction of dental pulp cells and an increase in intercellular space were noted. No marked floating cells and cell death were observed (Fig. 2B). Exposure of pulp cells to 0.1 mM BisGMA for 26-h led to marked cell retraction and decrease in cell density. Some cells became round and even floated in the culture medium (Fig. 2C).

3.3. Stimulation of PGE_2 production, COX-2 mRNA and protein expression in dental pulp cells by BisGMA

Exposure of dental pulp cells to 0.1 mM BisGMA markedly stimulated the COX-2 mRNA expression as analyzed by RT-PCR (Fig. 3A). Accordingly, BisGMA (0.1 mM) also evidently elevated the COX-2 protein expression in dental pulp cells as evaluated by western blotting (Fig. 3B). A 26-h exposure of pulp cells to higher concentrations of BisGMA stimulated PGE_2 production (Fig. 3C). At concentrations of 0.1 and 0.2 mM, BisGMA elevated the PGE_2 levels of pulp cells to 2-fold and 3.5-fold of control, respectively.

3.4. Effect of aspirin on BisGMA-induced PGE_2 production and cytotoxicity in dental pulp cells

Aspirin, a COX-1 and COX-2 inhibitor, inhibited the basal level of PGE_2 production in control dental pulp cells. Moreover, aspirin (100 & 200 μM) completely prevented the BisGMA (0.1 mM)-induced elevation of PGE_2 production in dental pulp cells (Fig. 4A). However,

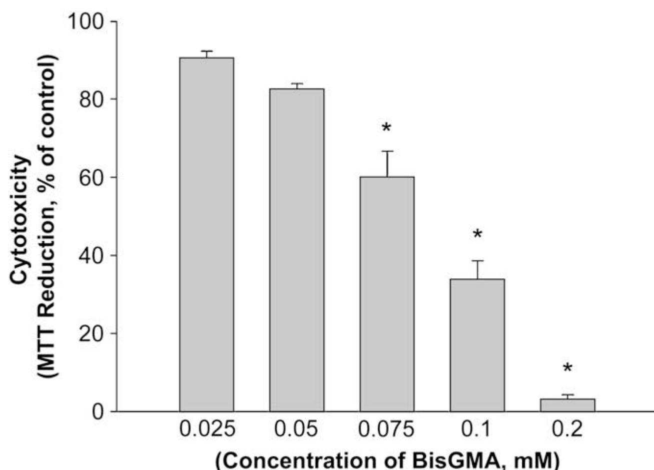

Fig. 1. Cytotoxicity of BisGMA to dental pulp cells. Dental pulp cells were exposed to solvent (control) or various concentrations of BisGMA for 26-h. Viable cell numbers were estimated by MTT assay. Results were expressed as percentage of control (% of MTT reduction) ($n = 3$). *Denotes marked difference ($p < 0.05$) when compared with control.

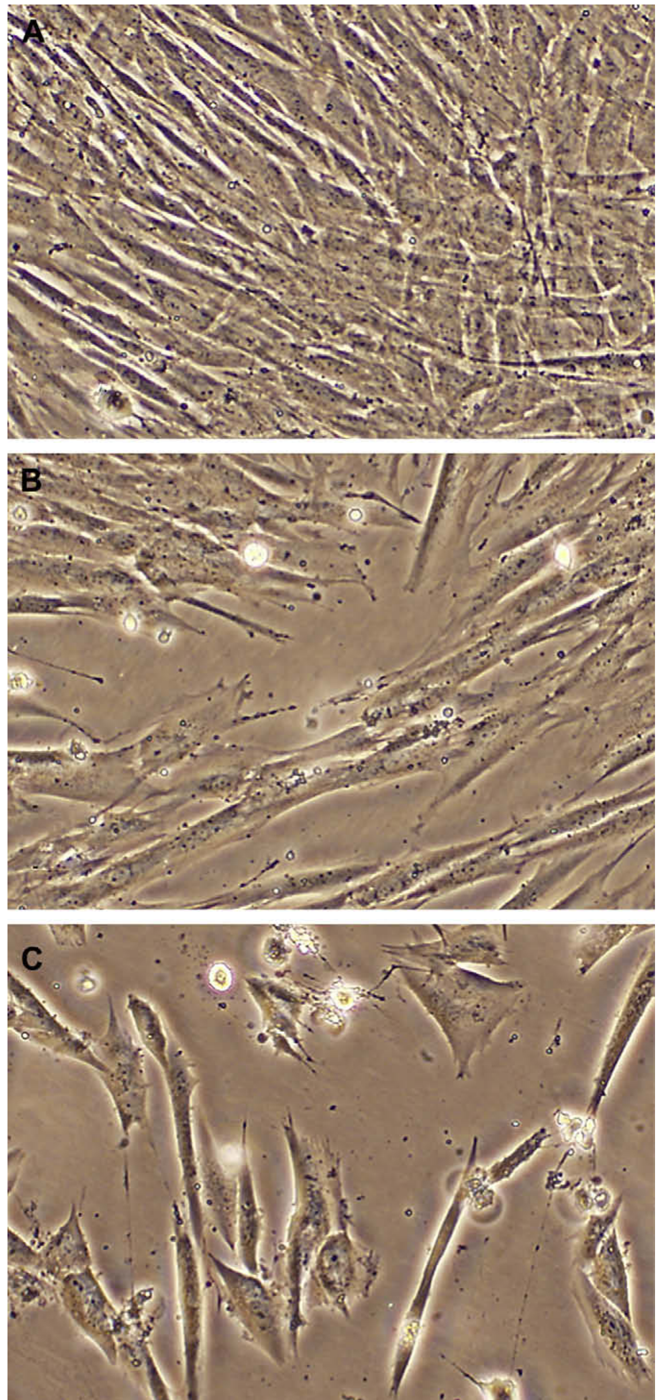

Fig. 2. Morphological changes of dental pulp cells after 24-h exposure to BisGMA. (A) Control pulp cells, (B) pulp cells exposure to 0.075 mM BisGMA for 26-h, (C) pulp cells exposure to 0.1 mM BisGMA for 26-h. 100×, original magnification. One representative picture was shown.

pretreatment by aspirin showed little effect on BisGMA-induced cytotoxicity to dental pulp cells (Fig. 4B).

3.5. ROS production and its role in BisGMA-induced COX-2 expression and PGE_2 production in dental pulp cells

Exposure of dental pulp cells to BisGMA (0.05 and 0.1 mM) markedly elevated the cellular ROS levels within 3-h of exposure (Fig. 5A). A 26-h exposure to 0.05 and 0.1 mM of BisGMA increased

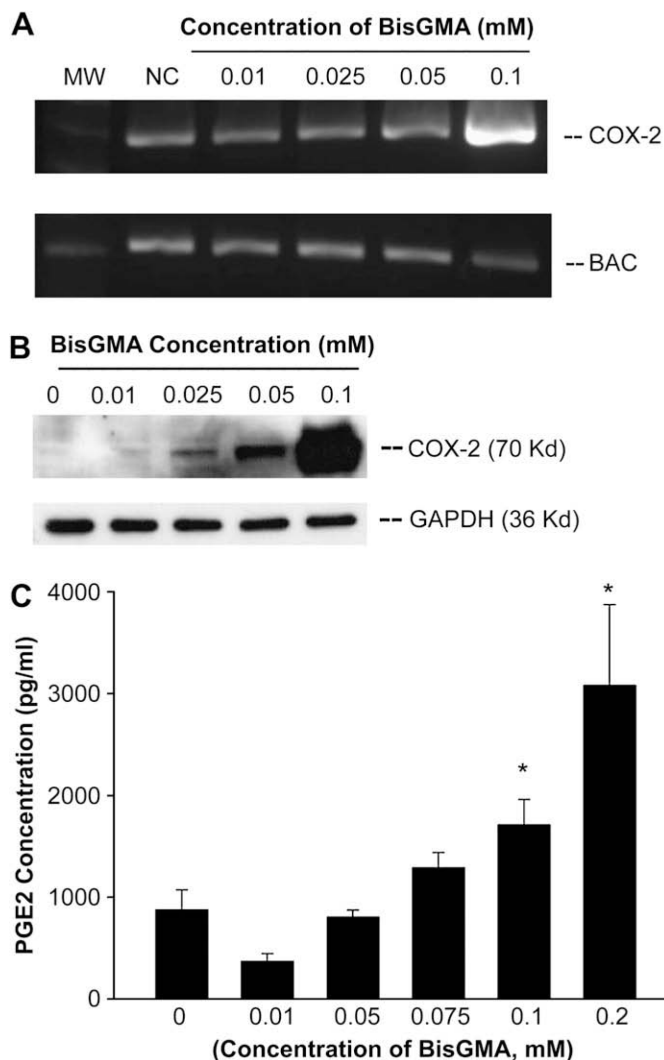

Fig. 3. Stimulation of COX-2 mRNA and protein expression as well as PGE_2 production in dental pulp cells by different concentrations of BisGMA. (A) Expression of COX-2 mRNA in control pulp cells (NC) and pulp cells after exposure to BisGMA (0.01–0.1 mM) as analyzed by RT-PCR, (B) COX-2 protein level of pulp cells after exposure to BisGMA (0.01–0.1 mM) as analyzed by western blotting. One representative picture was shown. (C) PGE_2 production of pulp cells after exposure to different concentrations of BisGMA ($n = 5$). *Denotes significant difference when compared with untreated control ($p < 0.05$).

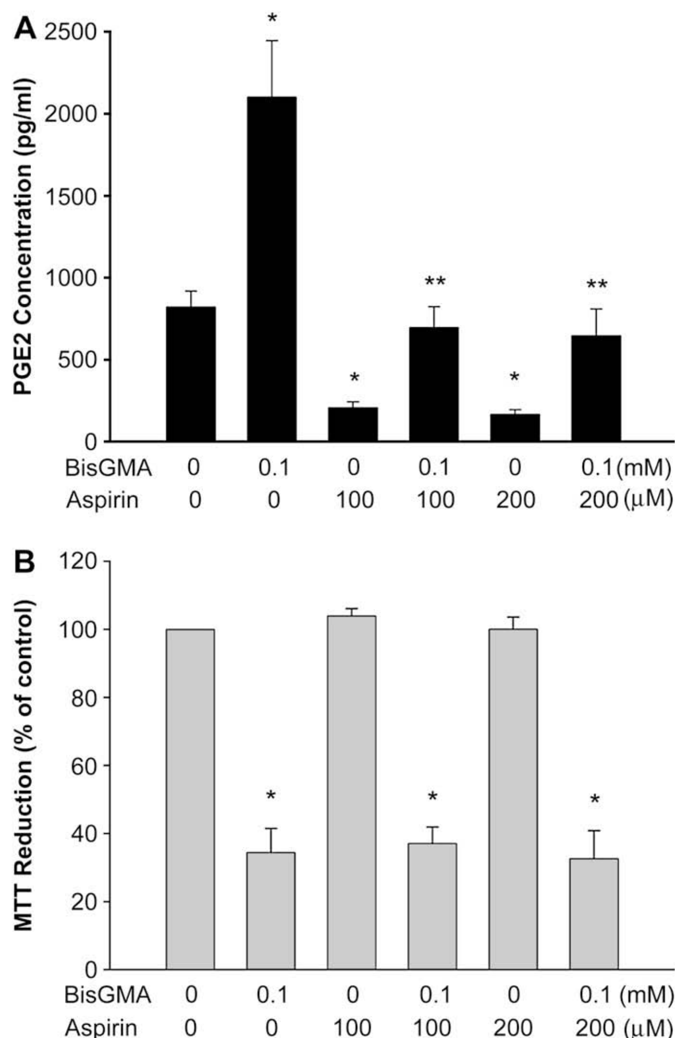

Fig. 4. (A) Effect of aspirin on BisGMA-induced PGE2 production in dental pulp cells. Results were expressed as Mean ± SE ($n = 9$), (B) effect of aspirin on BisGMA-induced cytotoxicity (MTT reduction, % of control) to dental pulp cells ($n = 3$). *Denotes significant difference when compared with solvent-treated control. **Denotes significant difference when compared with 0.1 mM BisGMA-treated group.

ROS levels by 30% and 60% as indicated by increases in cellular DCF fluorescence (Fig. 5B). One critical question is whether ROS production is crucial for induction of COX-2 by BisGMA in dental pulp cells. We interestingly found that BisGMA elevated COX-2 mRNA expression in dental pulp cells and this event can be attenuated by catalase (1000 U/ml, a H_2O_2 degrading enzyme) and NAC (5 mM, a general free radical scavenger) (Fig. 5C). Consistently, catalase (500 and 1000 U/ml) also prevented the BisGMA-induced elevation of PGE_2 production in dental pulp cells (Fig. 5D).

3.6. Role of MEK/ERK signaling in BisGMA-induced PGE_2 production

BisGMA (0.1 mM) activated ERK1/2 in pulp cells within 60 min of exposure as indicated by elevation of ERK1/2 phosphorylation. No marked difference in control protein, GAPDH, was noted (Fig. 6A). Since activation of ERK1/2 is regulated by upstream MEK1/2 signaling, we tested whether BisGMA-induced PGE_2 production was mediated via MEK/ERK signaling. Intriguingly, we found that pretreatment by U0126 (20 μM) for 30 min markedly decreased the BisGMA-induced phosphorylation of ERK (Fig. 6B). Accordingly, U0126 (10 and 20 μM) at popularly-used concentrations suppressed the basal level of PGE_2 production in dental pulp cells. In addition, the BisGMA-induced elevation of PGE_2 production in dental pulp cells was significantly attenuated by U0126 (10 and 20 μM) (Fig. 6C).

3.7. Effect of catalase and U0126 on BisGMA-induced cytotoxicity to pulp cells

We further tested whether the cytotoxicity of BisGMA to dental pulp cells is related to ROS production and MEK/ERK signaling. Morphologically, BisGMA induced marked cell retraction of pulp cells (Fig. 7B). Pretreatment and co-incubation with catalase (500 U/ml) effectively prevented the BisGMA-induced morphological alterations. Pulp cells retained the spindle-shaped appearance of fibroblasts (Fig. 7D). Accordingly, quantitative MTT assay also confirmed that catalase (500 and 1000 U/ml) completely prevented the BisGMA-induced cytotoxicity to pulp cells (Fig. 7E). However, U0126 (10 and 20 μM) was not able to protect pulp cells from BisGMA cytotoxicity (Fig. 7F).

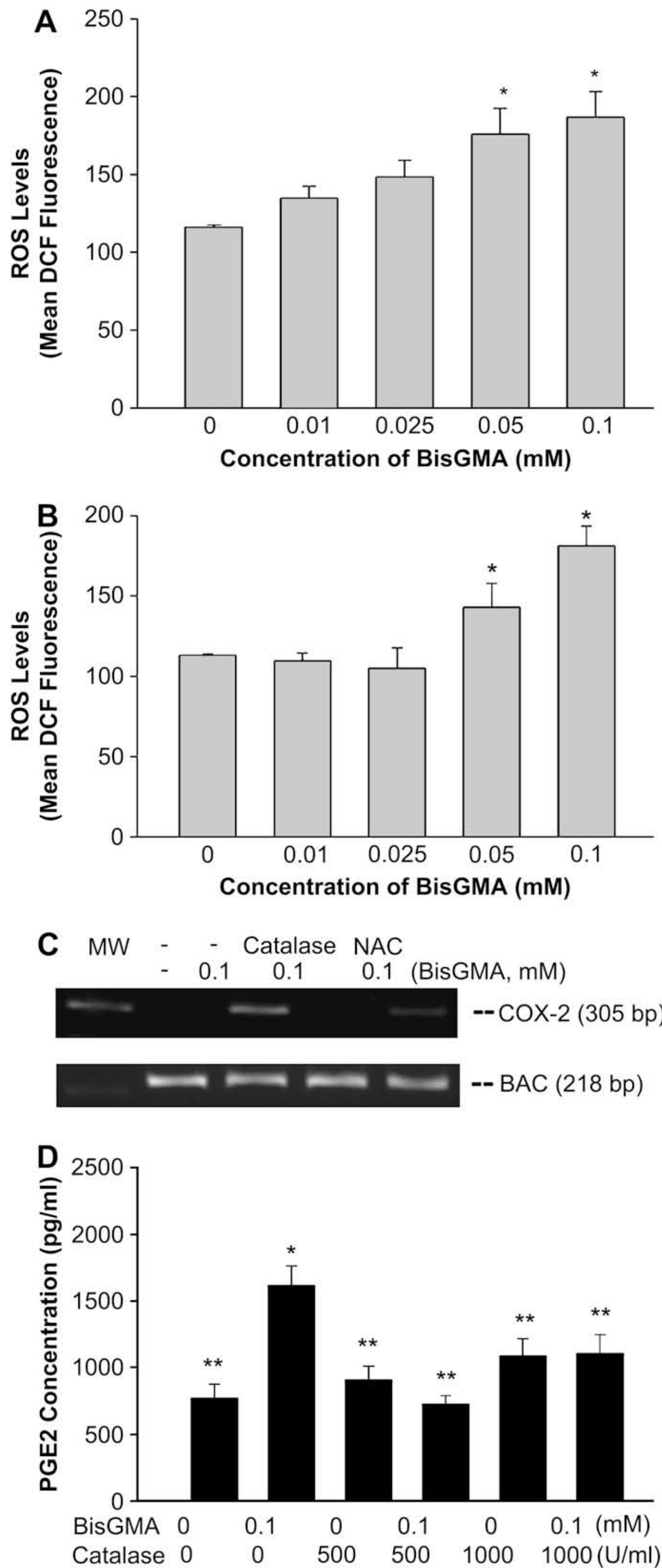

Fig. 5. Induction of ROS production in dental pulp cells by BisGMA. The exposure of dental pulp cells to different concentrations of BisGMA for (A) 3-h ($n = 8$) and (B) 26-h ($n = 6$), induced intracellular ROS production as indicated by elevation of cellular DCF fluorescence. Mean DCF fluorescence was shown (Mean ± SE). (C) Catalase (1000 U/ml) and NAC (5 mM) prevented the BisGMA-induced COX-2 mRNA expression, (D) catalase attenuated the BisGMA-induced PGE_2 production in dental pulp cells ($n = 10$). *Denotes significant difference when compared with solvent-treated control. **Denotes significant difference when compared with 0.1 mM BisGMA-treated group.

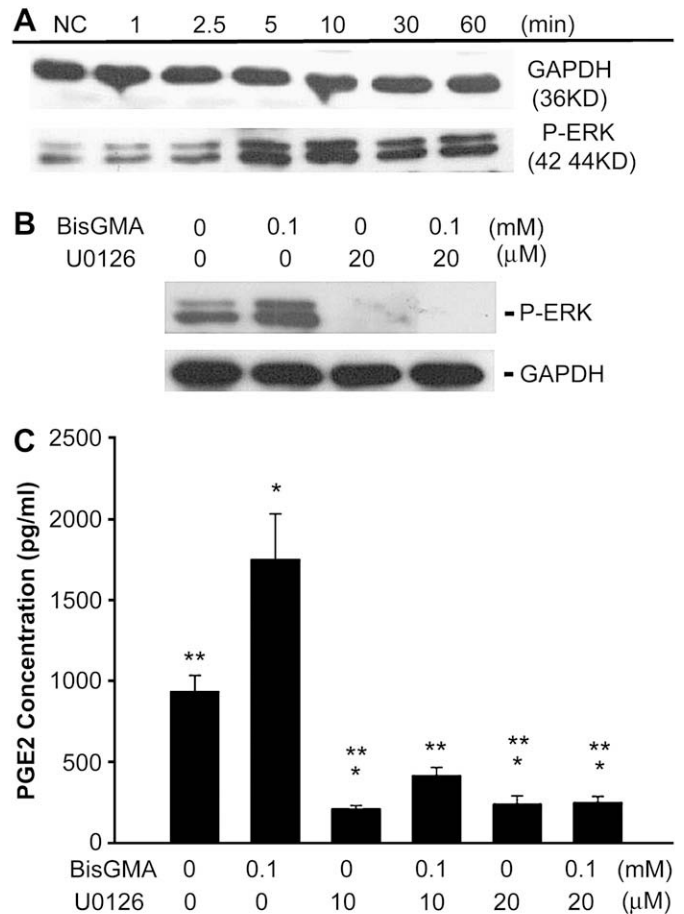

Fig. 6. (A) Stimulation of ERK phosphorylation by BisGMA (0.1 mM). Pulp cells were exposed to BisGMA for different time periods (1, 2.5, 5, 10, 30 and 60 min). Protein lysates were subjected to western blotting with antibody against p-ERK and GAPDH. One representative picture was shown. (B) Pulp cells were exposed to BisGMA for 10 min with or without pretreatment by U0126 (20 μM) for 30 min. The phosphorylation of ERK was evaluated by western blotting. (C) Pretreatment by U0126 (10 and 20 μM) inhibited the BisGMA-induced PGE_2 production in dental pulp cells ($n = 9$). *Denotes significant difference when compared with solvent-treated control. **Denotes significant difference when compared with 0.1 mM BisGMA-treated group.

4. Discussion

Composite resin and dentin adhesives are widely used for operative restoration of decayed teeth. However, restoration of tooth decay by these materials may potentially induce pulpal inflammatory response and even necrosis of dental pulp [7,8]. In additional to the presence of marginal leakage and residual bacterial invasion into the dental pulp, various monomers may potentially release from composite resin or dentin adhesives and affect the vitality and biological activities of dental pulp cells. The mechanistic insight for pulp inflammation after operative restoration is not well-elucidated. We proposed that this can be due to cytotoxicity and secondary inflammatory responses or direct stimulation of inflammatory mediator release of pulp cells by resin monomers in this study. Exposure of dental pulp cells to higher than 0.075–0.1 mM BisGMA leads to marked morphological changes and cytotoxicity. These results are generally similar to previous reports on the cytotoxicity of various resin monomers in different kind of cells [18,28–30]. Various monomers such as TEGDMA, HEMA, BisGMA can be eluted by water, methanol, and 75% ethanol/water solution *in vitro* [4–6]. Clinically the release of monomers occurs during degradation of resin materials by oxidative and hydrolytic enzymes in the saliva and pulp tissue or by reaction with other exogenous chemicals such as acid,

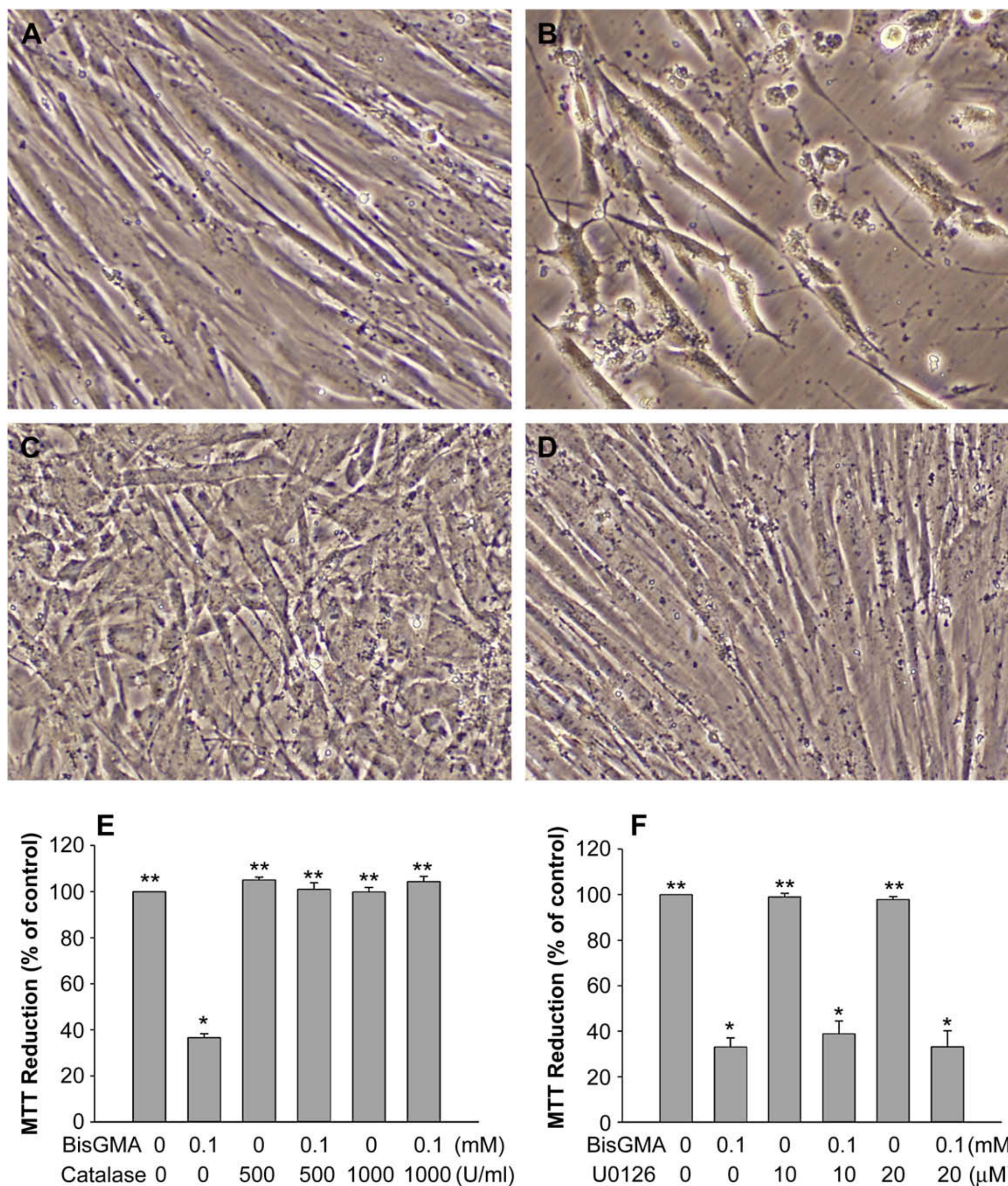

Fig. 7. Morphological changes of (A) control dental pulp cells, (B) 0.1 mM BisGMA-treated cells, (C) catalase (500 U/ml)-treated pulp cells, (D) catalase (500 U/ml) and 0.1 mM BisGMA-treated dental pulp cells. The cytotoxic effect (as MTT reduction, % of control) of 0.1 mM BisGMA and its prevention by (E) catalase (500 and 1000 U/ml) ($n = 4$) and (F) U0126 ($n = 4$). *Denotes significant difference when compared with solvent-treated control. **Denotes significant difference when compared with 0.1 mM BisGMA-treated group.

bases, salt, alcohols and oxygen [6,31]. It has been estimated that about 8–10% of unreacted monomers in dental composites are elutable and the elution reach complete within 1–3 days [6,32]. Much higher amount of eluted monomers was found when the composite resin is not adequately light-cured [33]. These released monomers or un-reacted monomers such as HEMA and TEGDMA are shown to diffuse through dentinal tubules in sufficient concentrations to cause tissue damage and inflammatory response [18]. While BisGMA is not readily soluble in water and available only in small amounts in a hydrophilic environment, BisGMA can be used as a representative acrylate compound for studying the toxic mechanisms of resin monomers on biological tissues.

A number of inflammatory mediators such as cytokines, substance P and prostaglandins (PGs) are suggested to contribute to pulpal inflammation [9–11,13]. Elevation of PGE_2 in dental pulp may increase vascular permeability and stimulate neutrophil recruitment [34]. However, various monomers cannot induce interleukin-1β release in gingival fibroblasts and HaCaT keratinocytes [30]. Interestingly we found that BisGMA may stimulate COX-2 mRNA and protein expression as well as PGE_2 production in dental pulp cells. PGE_2 may further stimulate calcium signaling of pulp cells via activation of prostaglandin EP receptors [14], thereby affect pulp cell functions. This may partly explain why restoration of tooth decay by dental adhesives and composite resins may induce pulpal inflammatory response *in vivo* [7,8]. Aspirin, a COX inhibitor, was able to attenuate the BisGMA-induced PGE_2 production, but not BisGMA-induced cytotoxicity. This further indicates that BisGMA-induced PGE_2 production was mediated by COX-2 stimulation. But

stimulation of COX-2 expression is not the major factor for BisGMA cytotoxicity to pulp cells.

Most of the resin monomers such as TEGDMA and HEMA have been shown to induce ROS production and contribute to cytotoxicity and genotoxicity [16–18]. Limited information is known about the role of ROS in mediating chemical-induced pulpal inflammation. Accordingly, we interestingly found that exposure of pulp cells to BisGMA (>0.05 mM) also elevated the intracellular ROS levels. Catalase, a H_2O_2 degradation enzyme, could prevent the BisGMA-induced PGE_2 production and cell death. Similarly NAC also suppressed the BisGMA-induced PGE_2 production. This suggests that BisGMA may induce COX-2 expression, PGE_2 production and cytotoxicity via generation of H_2O_2 or other ROS in dental pulp cells.

Recently oxidative stress has been shown to perpetuate inflammation, cell death and tissue degeneration via redox-sensitive signal transduction pathway [19,35]. HEMA may induce ERK activation but inhibit protein kinase B (Akt) activity, both of which may modulate the cytotoxicity to pulp cells by HEMA [36]. Inhibition of ERK and phosphatidylinositol 3 kinase/Akt signaling shows differential effects on the TEGDMA-induced cytotoxicity to pulp cells [37]. Induction of ROS by HEMA and TEGDMA may also activate ERK1/2, JNK and p38 to modulate the cytotoxicity and apoptosis of salivary gland cells [20]. Eckhardt et al. (2008) also found that TEGDMA may stimulate ROS production and induce DNA damage and activate ATM, p38 and ERK in THP-1 mononuclear cells to control cell survival and apoptosis [21]. Little is known about the mechanisms responsible for BisGMA cytotoxicity. We further tested whether BisGMA-induced COX-2 expression and PGE_2 production were mediated by ROS production and MEK/ERK1/2 signaling. Similar to other resin monomers, BisGMA rapidly induced ERK phosphorylation and activation in pulp cells within 60 min of stimulation. This event is mediated via upstream ROS production by BisGMA in dental pulp cells. Pretreatment of pulp cells with U0126, a MEK/ERK signaling inhibitor, markedly inhibited the BisGMA-induced ERK signaling and the downstream PGE_2 production. This suggests that BisGMA-induced COX-2 expression and PGE_2 production is mediated by ROS and MEK/ERK signaling. Unexpectedly, U0126 pretreatment was not able to prevent BisGMA cytotoxicity. This suggests that BisGMA induces ROS generation to mediate and cytotoxicity by pathways other than MEK/ERK.

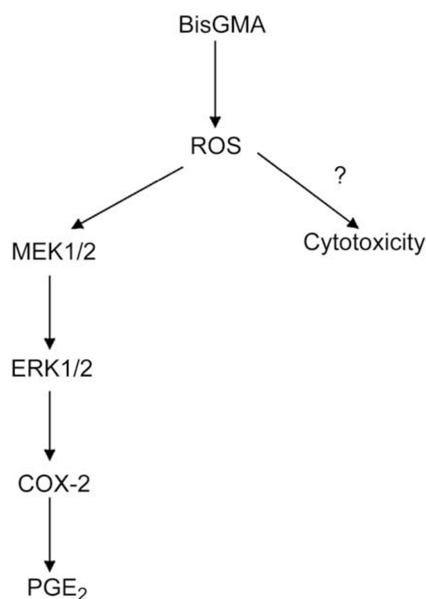

Fig. 8. Signal transduction pathways responsible for the BisGMA-induced COX-2 activation, PGE_2 production and cytotoxicity in human dental pulp cells.

5. Conclusion

We present the differential mechanisms responsible for the BisGMA-induced COX-2 expression, PGE_2 production and cytotoxicity in dental pulp cells. In conclusion, after polymerization of dental adhesives or composite resin, unpolymerized monomers such as BisGMA may potentially affect the biological activity of dental pulp especially when the caries cavity is deep, leading pulp necrosis or inflammatory responses. BisGMA may induce COX-2 expression and PGE_2 production via ROS production and MEK/ERK signaling. However, the BisGMA-induced cytotoxicity to pulp cells was mediated via ROS and signaling via pathways other than MEK/ERK1/2 (Fig. 8). Further studies on the upstream and downstream signaling pathways responsible for the induction of inflammatory mediators release and cytotoxicity by the resin monomers should be done to further highlight our understanding the pulpal responses to operative restoration.

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Appendix

Figures with essential colour discrimination. Figs. 2 and 7 in this article may be difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.04.034.

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