Cytotoxicities of a 4-META/MMA-TBBO Resin against Human Pulp Fibroblasts

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This study was designed to investigate the cytotoxicity of MMA (methyl methacrylate) and 4-META (4-methacryloyloxyethoxycarbonylphthalic anhydride)/MMA with or without TBBO (tri-*n*-butylborane partially oxide), an initiator and TBBO alone against human pulp fibroblasts (HPF).

Cell viabilities were measured by MTT assay. The cytotoxicity of 4-META/MMA-TBBO was comparable with that of MMA-TBBO. TBBO showed higher cytotoxicity than 4-META/MMA. The cytotoxicity induction of a 4-META/MMA-TBBO resin may be preferably associated with TBBO.

Key words: Cytotoxicity, 4-META/MMA-TBBO, Human pulp fibroblasts

INTRODUCTION

A 4-META/MMA-TBBO resin which is composed of 4-META, MMA and TBBO is widely used in dentistry as a bonding agents^{1–3)}. There have been many previous studies of cytotoxicity^{4–7)} and pulp responses^{8,9)}. Since high concentrations of 4-META are not obtained in water due to its hydrophobicity, some studies have shown an adverse effect of 4-META on pulp and cytotoxicity when this compound was solubilized at high concentrations in biological systems by methyl methacrylate or dimethyl sulfoxide^{4–8)}. We previously investigated the hemolytic activity of 4-META and 4-MET, a hydrolyzed product of 4-META, which suggested that the hemolytic activity of 4-MET was markedly smaller than that of 4-META¹⁰⁾. In a 4-META/MMA-TBBO resin, 4-META and TBBO are contained by 5% and 9.2% w/w in MMA, respectively. Therefore, the quantity of TBBO is about two-fold greater than 4-META. The molecular weight of TBBO, a partially oxidized TBB, is unknown, therefore, it is difficult to compare the cytotoxicity of TBBO with 4-META or MMA. To clarify the cytotoxicity of a 4-META/MMA-TBBO resin toward pulp, in the present study, we examined the cytotoxic activity of 4-META/MMA or MMA with TBBO and TBBO alone against HPF cells.

MATERIALS AND METHODS

Materials

Monomer (4-META/MMA, Lot. No. VG3) and catalyst (TBBO, Lot. No. VE 42) (Sun Medical Co. Ltd. Moriyama Japan) were used. MMA (Tokyo Kasei Kogyo Co.) was further purified. The following chemicals and reagents were obtained from the

indicated companies: fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS); Cell Titer 96 Aqueous One solution of MTS[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Promega Co. Madison, WI).

Cell culture

HPF cells were obtained from a 4-year-old female undergoing extraction of a supernumerary tooth. Informed consent was obtained from her parents. Ethical clearance for the study was obtained similarly to that previously reported¹¹⁾. The tissue was cut into 1 to 2 mm³ pieces, washed twice in phosphate-buffered saline (PBS) supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin, and placed into tissue culture dishes. The explants were incubated in culture medium consisting of α -MEM, 30% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin, at 37°C in a humidified atmosphere of 5% CO₂ in air. When outgrowth was observed in the cultures, the medium was replaced twice until the cells reached confluence. The cells were then detached from the monolayer by brief treatment with 0.05% trypsin/0.004 M EDTA and recultured in α -MEM containing 30% FBS until confluent monolayers were again obtained. HPF cells were maintained with α -MEM containing 10% FBS. Cells between the fifth and seventh passages were used in the experiments described below.

Cytotoxicity

HPF cells were seeded into 96-well plates at a density of 5×10^3 cells/well in 0.1 ml of MEM with 10% FBS and cultured at 37°C for 2 days. Before the addition of test materials, the cells were washed twice with serum free medium. A stock solution of indicated concentrations of 4-META/MMA with TBBO, MMA

with TBBO or TBBO was prepared in dimethyl sulfoxide (DMSO). Immediately after preparations, it was diluted with DMSO and added to the wells; and the cells were then incubated at 37°C for 24 hours. DMSO (1%)-treated cells served as the control. The number of viable cells was determined by the MTS colorimetric assay¹²⁾. The value of the control (the absorbance at 492 nm) is represented as a cell survival value of 100%.

Statistical analysis

Student's *t* test was used to assess the statistical significance of differences.

RESULTS

Cytotoxicity of MMA/4-META and MMA with or without TBBO and TBBO alone

Results are shown in Fig. 1. The 0.25% 4-META/MMA was significantly more cytotoxic than its 0.125% counterpart. The 0.125% 4-META/MMA was comparable with the control. There was no significant difference in the cytotoxicity between MMA/4-META and MMA with TBBO. In contrast, the cytotoxicity of TBBO was significantly greater than that of 4-META/MMA or MMA with TBBO at each concentration level. That of 0.5% TBBO corresponded to approximately 50% viability.

DISCUSSION

For approximately 30 years, 4-META/MMA-TBBO resin has been investigated¹⁻³⁾, but its characterization and biocompatibility are not fully understood.

Fig. 1 The cytotoxicity of 4-META/MMA with or without TBBO and MMA with TBBO at different concentrations against human pulp fibroblasts (HPF). Bars indicate the mean $\pm$ SD ($n=8$). Results were analysed by Student's *t*-test. A significant difference of 4-META/MMA between 0.125% and 0.25% ($p<0.001$) and that between TBBO and 4-META/MMA with TBBO ($p<0.05$) or MMA with TBBO ($p<0.05$) at the indicated concentrations was found. Methods are described in the text.

To clarify the stability of 4-META in aqueous media our previously study suggested that 4-META was rapidly converted to 4-MET, a hydrolyzed product of 4-META, in deuterium oxide using NMR spectroscopy¹⁰⁾. These findings agreed with those of spectroscopic experiments¹³⁾ using laser-Raman spectra of 4-META in MMA solution. These findings suggested that 4-META with a carboxylic acid anhydride moiety is highly sensitive to moisture and is immediately converted to 4-MET with two carboxylic groups under clinical conditions even when carboxylic acid anhydride is retained until use.

We previously reported the strong hemolytic activity of 4-META and its large thermotropic alterations of L- α -dipalmitoylphosphatidylcholine liposomes as a model for biomembranes¹⁰⁾. However, the present study demonstrated that the cytotoxicity of 4-META/MMA with TBBO was comparable with that of MMA with TBBO, suggesting that 4-META may not affect the cytotoxicity induction. In contrast, TBBO showed greater cytotoxicity than 4-META/MMA and hence, the cytotoxic activity of 4-META/MMA-TBBO may be responsible for TBBO. The cytotoxicity reduction of 4-META in the medium may be due to the fact that 4-META is rapidly converted to 4-MET in water¹⁰⁾ and from that 4-META preferably interacts with proteins^{14,15)} and alkaline earth salts¹⁷⁾.

The nerve regeneration and proliferative activity in amputated pulp tissue after the application of a 4-META/MMA-TBBO resin were previously reported⁹⁾. This indicated that the healing activity of 4-META resin application was the same as that of calcium hydroxide, possibly due to boric acid derived from the decomposition of TBBO. From this it was clear that TBBO might be associated with much more pulp toxicity than 4-META.

The toxicity¹⁸⁾, cytotoxicity¹⁹⁾ and hemolytic activity²⁰⁾ of methacrylates are correlated with their hydrophobicity. In previous study²¹⁾, the values of hydrophobicity ($\log P_{\text{HPLC}}$) for MMA, TBBO and 4-META were 0.79, 0.005, and 2.13 (and 1.23), respectively, using an HPLC. These values are related to those of octanol-water partition coefficient ($\log P$). MMA with 0.76 is dissolved by 1-1.3% w/v in water at room temperature. Despite that MMA is a volatile substance, this compound is relatively water-soluble, however, 50% cytotoxicity of MMA against HPF cells was about 30 mM in this study, suggesting less cytotoxicity against HPF cells. Less cytotoxicity for MMA against human gingival fibroblasts (HGF) was previously reported²¹⁾. In contrast, 4-META shows two values of $\log P_{\text{HPLC}}$, suggesting that 4-META is 2.13, whereas 4-MET is 1.23. As above mentioned, 4-META is probably converted to 4-MET in water, resulting from the study of HPLC. Thus, in the present study, the cytotoxicity for 4-META/MMA or MMA with or without TBBO

was compared. Also, 1% DMSO was used to dissolve test compounds and hence, the survival (%) was represented as the control=100.

On the other hand, it was previously reported that the pulp-biocompatibility of 4-META/MMA-TBBO resins was responsible for the dentin-adhesive activity of this resin^{2,17}. The risks of pulpal toxicity of resin components are connected with their ability to diffuse through the dentine and accumulate in the pulp²³. The present studies are out of the scope of this problem but we have recently found the reduction of cytotoxic activity of 4-META/MMA in the presence of fetal bovine serum and human albumin (data not shown), possibly due to its interaction between 4-META/MMA and proteins.

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