Acta Biomaterialia 10 (2014) 722–731

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Urethane dimethacrylate induces cytotoxicity and regulates cyclooxygenase-2, hemeoxygenase and carboxylesterase expression in human dental pulp cells

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ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form 28 September 2013
Accepted 10 October 2013
Available online 17 October 2013

Keywords:
Dental pulp
Inflammation
Prostanoids
Resin monomers
Urethane dimethacrylate

ABSTRACT

The toxic effect of urethane dimethacrylate (UDMA), a major dental resin monomer, on human dental pulp is not fully clear. In this study, we investigated the influence of UDMA on the cytotoxicity, cell cycle distribution, apoptosis and related gene expression of dental pulp cells. The role of reactive oxygen species, hemeoxygenase-1 (HO-1) and carboxylesterase (CES) in UDMA cytotoxicity, was evaluated. UDMA induced morphological changes of pulp cells and decreased cell viability by 29–49% at concentrations of 0.1–0.35 mM. UDMA induced G0/G1, G2/M cell cycle arrest and apoptosis. The expression of cdc2, cyclinB1 and cdc25C was inhibited by UDMA. Moreover, UDMA stimulated COX-2, HO-1 and CES2 mRNA expression of pulp cells. The cytotoxicity of UDMA was attenuated by N-acetyl-L-cysteine, catalase and esterase, but was enhanced by Zn-protoporphyrin (HO-1 inhibitor), BNPP (CES inhibitor) and loperamide (CES2 inhibitor). Exposure of UDMA may potentially induce the inflammation and toxicity of dental pulp. These findings are important for understanding the clinical response of human pulp to resin monomers after operative restoration and pulp capping, and also provide clues for improvement of dental materials.

1. Introduction

Nowadays, methacrylate-based resin materials are ubiquitous in dental therapy. Besides restoring impaired tooth structure, they are also used in dental prosthesis fabrication, endodontic treatment, caries prevention and even oral surgery [1]. The utilization and function of resin materials are accomplished by polymerization. However, monomer–polymer conversion is never complete. A considerable amount of methacrylate monomer may be released from these materials, diffuse into the oral environment and probably induce adverse biological reactions [2]. The depicted harmful events include macroscopically local and systemic toxicity, such as pulpal damage and estrogenic effects [3,4], and microscopically genetic and cellular toxicity [5]. The most often detected components leached from dental resin materials include 2-hydroxyl ethylmethacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), bisphenol A-diglycidyl dimethacrylate (BisGMA) and urethane dimethacrylate (UDMA) [6]. However, the studies concerning UDMA-induced toxicity are relatively limited [7,8]. UDMA is one of the most commonly used monomers. As a multifunctional ester with aliphatic urethane chain, it provides elasticity and more space for inorganic filler incorporation. Compared to aromatic-based monomers, such as BisGMA, UDMA is less viscous and thus easier for clinical manipulation, and can construct material with better flexural and tensile strength [7,9]. These properties have made UDMA more popular for use in various applications, such as materials for caries restoration and dentin bonding. Nonetheless, the urethane chain polymer absorbed more water than the aromatic base network and was more susceptible to hydrolytic degradation and therefore leached into the oral aqueous environment [10].

Direct pulp capping and indirect pulp therapy to deep caries with dentin a bonding agent (DBA) or resin composite have been reported to cause impaired healing, inflammation and necrosis of human pulp tissue [11–13]. As one of the main leached ingredients, UDMA is assumed to contribute to these consequences. UDMA has been reported to suppress vitality and alter cellular differentiation ability and metabolic activity for several types of cells [14–16]. However, research addressing dental pulp cells, which are the main affected targets, is still scarce, and the underlying mechanism is far from clear. Cytotoxicity of TEGDMA, BisGMA and

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http://dx.doi.org/10.1016/j.actbio.2013.10.006

HEMA has been found to be related to cell cycle deregulation [17–20]. Our recent study reported UDMA-induced cytotoxicity and cell cycle disturbance in Chinese hamster ovary cells (CHO-K1 cells), but the underlying mechanism still waits for elucidation [21]. Expressions of some genes like cyclin B1, cdc2, cdc25C and p21 are critical for cell cycle progression and apoptosis [22]. Whether and how UDMA might induce cell cycle interruption in dental pulp cells, and the expressions of the correlated factors, should be meaningful issues to survey.

Chemical toxicity may be induced by an imbalance of redox homeostasis. Excessive reactive oxygen species (ROS) have been found to contribute to TEGDMA, HEMA and BisGMA toxicity and associate with cell cycle deregulation, apoptosis and necrosis [17–19]. Hemeoxygenase-1 (HO-1) is an oxidative stress response gene with actions for promoting cell survival, inflammation resolution and circulatory integrity [23]. Its expression is modulated by changes in cellular redox potential that are often provoked by ROS production or glutathione depletion. It has been reported that H_2O_2 as a potent ROS may induce HO-1 expression in human pulp cells [24]. By the induction of oxidative stress, BisGMA and HEMA were found to enhance HO-1 expression in human pulp cells and mouse macrophages, respectively [18,25]. Whether UDMA induces HO-1 expression and the role of HO-1 in UDMA-induced toxicity to pulp cells were interesting issues to explore. As previously mentioned, pulpal inflammation is a common result from the application of resin materials on or near pulp tissue. Upon pulpal inflammation, the level of inflammatory mediators such as prostaglandin E_2 (PGE_2) and PGF2α elevated [26]. As prostanoids are synthesized from arachidonic acid by cyclooxygenase (COX), and the inducible form, e.g. COX-2, is crucial for inflammatory response. Therefore, the influence of UDMA on the expression of COX-2 in dental pulp cells awaits investigation.

Carboxylesterase (CES), which belongs to the class of serine hydrolases, is capable of hydrolyzing a wide variety of xenobiotics with ester, thioester and amide groups [27]. This enzyme is differentially distributed in human tissues, and most abundantly in liver. As it is recognized as catalyzing aromatic and aliphatic esters, CES is proposed to contribute to the degradation and metabolism of UDMA, which is an ester of aliphatic urethane alcohol and methacrylic acids, in human tissue. CES distribution in human pulp cells was limited until recently, and the gene expression was found to be induced by BisGMA [28]. We would like to know if UDMA can induce CES production in dental pulp cells, and the role of esterase in regulation of UDMA-induced toxicity.

The aim of this study is to investigate the influence of UDMA on the cytotoxicity, cell cycle distribution, apoptosis and related genes expression of human dental pulp cells. The roles of ROS, COX-2, HO-1 and CES in UDMA toxicity were evaluated. Elucidating these issues may help to gain insight into the mechanism of disappointing clinical response and may provide hints for improvement.

2. Materials and methods

2.1. Chemicals and reagents

Cell culture medium and reagents were obtained from Gibco Laboratories (Life Technologies, Grand Island, NY, USA). UDMA, dimethyl sulfoxide (DMSO), 3-(4,5-dimethyl- thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT), catalase, N-acetyl-L-cysteine (NAC), porcine esterase, bis(p-nitrophenyl) phosphate (BNPP), zinc protoporphyrin (Zn-P) and propidium iodide (PI) were purchased from Sigma-Aldrich (Sigma Chemical Company, St Louis, MO, USA). UDMA was dissolved in DMSO as a stock solution then sequentially diluted in the culture medium for use. The final concentration of DMSO in the culture medium was less then 0.5% (v/v). Loperamide was from Tocris (Tocris Cookson Ltd, Northpoint Fourth Way Avonmouth, UK). Reagents for flow cytometry were manufactured by Becton Dickinson (San Jose, CA, USA). Total RNA isolation kits were bought from Qiagen (Qiagen Company, Taiwan). Polymerase chain reaction (PCR) primers were synthesized by Genemed Biotechnologies, Inc. (San Francisco, CA, USA). Mouse anti-human cyclin B1, cdc2, cdc25c, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and rabbit anti-human COX-2 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Culture of primary human dental pulp cells

With approval of the Research Ethics Committee of National Taiwan University Hospital, premolars or third molars were acquired from healthy young persons with proper informed consent. An explant technique was executed quickly to cultivate dental pulp cells as described before [18,19,28]. In brief, the pulp stump was first taken out from the tooth then cut into tiny pieces ($\sim$1 mm^3) with a surgical scalpel. The tissue slices were then cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), penicillin and streptomycin at 37 °C in a humidified atmosphere of 95% air–5% CO_2. The cells outgrown from the tissues were trypsinized, collected and then subcultured in the same condition. In the following studies, dental pulp cells from subculture passage numbers 3 to 8 were used.

2.3. Cytotoxicity of UDMA on of dental pulp cells

1×10^5 dental pulp cells were seeded into each well of a 24-well culture plate containing DMEM with 10% FBS, and were cultured for 24 h to allow adhesion. After that, the medium was changed to a fresh one containing solvent control and various concentrations of UDMA (final concentration of 0 to 0.35 mM), and the cells were further cultured for 24 h. Finally, cells were rinsed with phosphate buffered saline (PBS) and incubated in DMEM with 0.5 mg ml^{-1} of MTT for 2 h. The generated formazan product by viable cells was dissolved in DMSO and read against blank reagent at OD_{540} using a Dynatech Microwell plate reader (Dynatech Labs Inc., Chantilly, VA, USA).

2.4. Effect of UDMA upon the cell cycle distribution in dental pulp cells

Briefly 2.5×10^5 cells were plated into each well of 6-well culture dishes and incubated with DMEM containing 10% FBS. After 24 h, solvent control and different concentrations of UDMA were added (final 0 to 0.35 mM) and cells were further cultivated for 24 h. DNA content of individual cell was then analyzed by flow cytometry. Floating and attached cells were all gathered in a centrifuge tube, re-suspended and fixed in 70% ice-cold ethanol containing RNase (2 mg ml^{-1}) for 30 min. After rinsing with PBS, the cells were finally stained with PI (40 μg ml^{-1}) for 10 min at room temperature. A FACSCalibur Flow Cytometer (Becton Dickinson, Worldwide Inc., San-Jose, CA, USA) equipped with an argon laser was used to detect and gauge the PI-elicited fluorescence from a single cell. The wavelength of laser excitation was set at 488 nm and the emission collected was set at greater than 590 nm. The FL2 fluorescence was measured in a linear/log scale mode. A total of 1×10^4 cells were analyzed for each sample. The percentage of cells in sub-G0/G1, G0/G1, S and G2/M phase was calculated using ModFit and CELL QUEST programs. Alteration of cell morphology was inspected and photographed under a phase contrast microscope (Olympus IX71, Olympus America Inc.).