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Effects of antioxidants on DNA double-strand breaks in human gingival fibroblasts exposed to dental resin co-monomer epoxy metabolites

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ABSTRACT

Objective. Eluted dental resin co-monomers can be metabolized to intermediate methacrylic acid (MA) and, further, to epoxy metabolites. Antioxidants have been studied previously, with the intention of decreasing the DNA double-strand breaks (DNA-DSBs) in human gingival fibroblasts (HGFs). In this study, the effects of the antioxidants, ascorbic acid (Asc) and N-acetylcysteine (NAC), were investigated on co-monomer metabolite-induced DNA-DSBs.

Methods. HGFs were incubated with MA, 2,3-epoxy-2-methyl-propionicacid-methylester (EMPME) and 2,3-epoxy-2-methylpropionic acid (EMPA), respectively, in the presence or absence of antioxidants (Asc or NAC). EC_{50} Values were obtained from an XTT-based viability assay. DNA-DSBs were determined using a γ-H2AX assay.

Results. The cytotoxicity of the compounds could be ranked in the following order (mean ± SEM; n = 4): EMPA > EMPME > MA. The average number of DSBs-foci/cell induced by each substance at EC_{50}-concentration could be ranked in the following order (mean ± SD; n = 4): EMPA > EMPME > MA. EMPA (1.72 mM) and EMPME (2.58 mM) induced the highest number of DSBs-foci, that is 21-fold and 13-fold, respectively, compared to control (0.48 ± 0.08 foci/cell). The addition of Asc (50; 100; 200 μM) or NAC (50; 100; 200; 500 μM) to MA (15.64; 5.21 mM), EMPME (2.58 mM), and EMPA (1.72; 0.57 mM) significantly reduced the number of foci/cell in HGFs. The highest reduction could be found in HGFs with 1.72 mM EMPA, the addition of NAC (50; 100; 200; 500 μM) induced a 15-fold, 17-fold, 14-fold and 14-fold lower number of DSBs-foci/cell, respectively.

Significance. Dental co-monomer epoxy metabolites, EMPME and EMPA, can induce DNA-DSBs. The addition of antioxidants (Asc or NAC) leads to reduction of DNA-DSBs, and NAC leads to more prominent reduction of DNA-DSBs compared to Asc.

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1. Introduction

The unpolymerized co-monomers triethylene glycol dimethacrylate (TEGDMA) and 2-hydroxyethyl methacrylate (HEMA) can be released from incompletely polymerized composite resins [1], and thereby affect the activity of dental pulp cells or enter the intestine by swallowing, subsequently reaching the circulatory system and organs [1,2]. Our previous studies have demonstrated the uptake, distribution and elimination of radiolabeled ^{14}C-TEGDMA and ^{14}C-HEMA in guinea pigs [3,4]. As a result, the metabolism of TEGDMA and HEMA was postulated, and the formation of methacrylic acid (MA), a metabolisation intermediate of TEGDMA and HEMA, was described [3,4]. MA can be metabolized by two different pathways [5]. In one pathway (epoxide pathway), it was suggested that 2,3-epoxy-2-methyl-propionicacid-methylester (EMPME) might be formed [6]. Additionally, the C-C-double bond of MA can be oxidized, consequently, the epoxy metabolite, 2,3-epoxy-2-methylpropionic acid (EMPA) can be formed [6–8]. In this process, hydrogen peroxide is involved as chemical catalyst [9], and cytochrome P450 2E1 (CYP2E1) also plays an important role [7]. In a previous study, it was shown that ^{14}C-TEGDMA and ^{14}C-HEMA are mainly metabolized via epoxide pathway in A549 cells [10], and the formation of EMPA in human oral cells (for example, human gingival fibroblasts (HGF) and human pulp fibroblasts (HPF)) has also been demonstrated [7].

In a previous study, the toxicology of EMPME and EMPA was investigated by the use of a modified fluorescent stem-cell test; as a result, the teratogenic effect was observed for EMPA, and an embryotoxic effect was observed for EMPME on the embryonic stem cells of mice [6]. A similar genotoxicity of epoxides was also found in glycidamide, the epoxy metabolite of acrylamide, which is commonly present in fried food [11], is highly reactive toward DNA by formation of covalent adducts on the N7-position of guanine, N3-position of adenine and N1-position of deoxyadenosine [12]. Since the glycidamide has an epoxy structure similar to those of EMPME and EMPA, it is likely that they will lead to a similar genotoxicity. Since the DNA damage can lead to carcinogenic and mutagenic effects [13], the epoxides are considered to be highly reactive molecules and toxic agents [8]. If they are left unrepaired, they can lead to cell death; chromosomal translocations and genomic instability may occur if they are misrepaired [14].

Many studies have dealt with the toxicology of co-monomers such as TEGDMA and HEMA, which can induce DNA-DSBs [15,16]. Schweikl et al. demonstrated that HEMA-induced apoptosis is a response to DNA damage [17]. However, in comparison with the precursors, TEGDMA, HEMA and the intermediate MA, whether the epoxy metabolites can induce more DNA-DSBs is still unknown. In this study, therefore, the effect of the co-monomer epoxy metabolites, EMPME and EMPA, on the DNA-DSBs, was investigated. In some studies, it has been demonstrated that the addition of antioxidants, such as Asc or NAC, can reduce the cytotoxic effects and DNA-DSBs of dental resin co-monomers [18–20]. It is not known whether antioxidants can lead to the reduction of DNA-DSBs in the presence of co-monomer epoxy metabolites. Therefore, in this study, the effects of Asc and NAC on the epoxide-induced DNA-DSBs in HGFs were also investigated.

2. Methods

2.1. Chemicals

EMPME and MA were obtained from Sigma–Aldrich (Weinheim, Germany). EMPA was synthesized by oxidation of MA, according to the method described by Yao and Richardson [9]. For the determination of cytotoxic effects, a cell-proliferation kit II from Roche Diagnostics (Mannheim, Germany) was used. Asc was purchased from Sigma–Aldrich (St. Louis, MO, USA), NAC was obtained from Alfa Aesar GmbH (Karlsruhe, Germany). Hydrogen peroxide (H_2O_2) was obtained from Sigma–Aldrich (Steinheim, Germany). MA, EMPME, EMPA, Asc and NAC were dissolved directly in the medium. All chemicals and reagents were of the highest purity available.

2.2. Cell culture

HGFs were obtained from Provitro GmbH (Berlin, Germany). The HGFs (passage 10) were cultured as described in our former study [15].

2.3. XTT-based viability assay

An XTT-based cell viability assay was used to determine the half-maximum effect concentration (EC_{50}) values for the investigated substances in HGFs. This assay was performed according to our previous study [15]. The cells were treated with medium containing MA (1–100 mM), EMPME (0.5–12 mM) and EMPA (0.01–10 mM), respectively, followed by incubation for 24 h. The formazan formation was quantified spectrophotometrically at 450 nm (reference wavelength 670 nm), using a microplate reader (MULTISKAN FC; Thermo Fisher Scientific (Shanghai) Instruments Co., Ltd., China). Four independent experiments were performed, each time in triplicate.

2.4. γ-H2AX immunofluorescence

DNA-DSBs formation was determined in HGFs by γ-H2AX assay, as described in our previous study [15]. In the following the procedure and modifications for the present study is outlined:

12 mm round cover slips (Carl Roth, Karlsruhe, Germany) were cleaned in 1 N HCl and distributed into a 24-well plate. HGFs were seeded at 7×10^4 cells/ml in each well with the medium, followed by overnight incubation at 37 °C. The cells were exposed for 6 h to medium containing the MA, EMPME, and EMPA, respectively, or the antioxidants alone; the concentrations of MA, EMPME and EMPA are determined by EC_{50}, $1/3EC_{50}$ and $1/10EC_{50}$, based on the XTT values: MA (15.64; 5.21; 1.56 mM), EMPME (2.58; 0.86; 0.26 mM), EMPA (1.72; 0.57; 0.17 mM), the concentrations of antioxidants tested alone were Asc (50; 100; 200; 500 μM) and NAC (50; 100; 200; 500 μM); these concentrations were based on a previous study [19]. Considering toxicity caused by 500 μM Asc from our result, the concentrations of antioxidants to be added to MA, EMPME, EMPA for γ-H2AX assay were: Asc (50; 100; 200 μM) and NAC (50; 100; 200; 500 μM). Negative control cells received the medium for 6 h. Positive control cells received 1 mM H_2O_2 in