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The effect of antioxidants on oxidative DNA damage induced by visible-light-irradiated camphorquinone/*N*,*N*-dimethyl-*p*-toluidine

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Abstract

Previous investigations have found that visible-light (VL)-irradiated camphorquinone (CQ), in the presence of a tertiary amine (e.g., *N*,*N*-dimethyl-*p*-toluidine, DMT), generates reactive oxygen species and causes oxidative DNA damage in vitro. In this study, oxidative DNA damage produced by VL-irradiated CQ/DMT, in the presence and absence of antioxidants (glutathione, *N*-acetyl-L-cysteine (NAC), mannitol, vitamin C, and vitamin E), was measured by the conversion of ΦX-174 RF I supercoiled (SC) double-stranded plasmid DNA into open and linear forms. VL-irradiated CQ/DMT, lacking antioxidant, damaged 99.4 ± 1% of the ΦX-174 RF I SC double-stranded plasmid DNA. Our results revealed that glutathione (10.0, 5.0, 2.5, 1.0, and 0.5 mM) and NAC (10.0, 5.0, and 2.5 mM) significantly ($p < 0.02$) reduced oxidative DNA damage produced by VL-irradiated CQ/DMT. Vitamin E, vitamin C, and mannitol were ineffective at reducing oxidative DNA damage produced by VL-irradiated CQ/DMT. Furthermore, vitamin E (10.0 and 5.0 mM) and vitamin C (10.0, 5.0, 2.5, 1.0, 0.5 mM) treatment significantly ($p < 0.02$) enhanced VL-irradiated CQ/DMT-induced oxidative DNA damage and caused significant ($p < 0.001$) DNA damage following VL-irradiation in the absence of CQ/DMT. As a result, future studies should evaluate whether glutathione and NAC effectively reduce or prevent oxidative damage induced by VL-irradiated CQ/DMT in vivo.

Keywords: Camphorquinone; Reactive oxygen species; Oxidative DNA damage; Antioxidants

1. Introduction

Numerous studies have revealed that resin-based dental filling materials (RBDF) release substances into the oral environment. For instance, it was found that 32 different organic substances leached from various RBDF materials [1]. Among these leachates were mainly unreacted (*co*)-monomers and initiating substances. Geurtsen et al. [2] showed that (*co*)-monomers (e.g., urethane di-methacrylate, UDMA, triethyleneglycol di-methacrylate, TEGDMA) and initiators (e.g., camphorquinone, CQ) were capable of producing moderate to severe cytotoxic reactions. Besides their cytotoxic effects, various organic leachates are genotoxic. Stanislawski et al. [3] reported that TEGDMA generated reactive oxygen species (ROS) in human fibroblasts, which accounted in part for its cytotoxic effects. It is known from several in vitro studies, which used isolated supercoiled (SC) plasmid DNA, that ROS damage DNA [4–6].

Although data exist describing the biocompatibility of RBDF and their leached organic components, most of the research has focused on the cytotoxicity and genotoxicity of (*co*)-monomers, such as TEGDMA and 2-hydroxy-ethyl-methacrylate (HEMA), largely because they leach in high concentrations. However, the potentially harmful effects of initiators are relatively under researched. It has been shown that CQ, one of the most widely used initiators in modern light-cured resin systems, leaches from all RBDF [1]. Atsumi et al. [7]

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found that CQ, in conjunction with a reducing agent (e.g., 2-dimethylaminoethyl methacrylate, DMA, or *N*,*N*-dimethyl-*p*-toluidine, DMT), exhibited cytotoxic activity in human oral cells. This study also revealed that visible-light (VL)-irradiated CQ/DMT generated ROS in high concentrations, which correlated with and was a likely mechanism for its cytotoxicity. Recently, Atsumi et al. [8] showed that VL-irradiated CQ/DMA was less cytotoxic than a VL-irradiated aromatic photosensitizer, 9-fluorenone (9F)/DMA, despite greater free radical production. They further showed that VL-irradiated 9F/DMA generated significantly more ROS in human pulp fibroblasts compared to VL-irradiated CQ/DMA. Only recently has the genotoxic potency of VL-irradiated CQ/DMT been demonstrated in vitro. Pagoria et al. [9] found that VL-irradiated CQ ± DMT produced significant DNA damage to ΦX-174 RF I SC double-stranded plasmid DNA in a concentration-dependent manner. They further revealed that VL-irradiated CQ/DMT generated significant amounts of ROS in vitro and suggested that ROS may be responsible for VL-irradiated CQ/DMT-induced oxidative DNA damage.

Numerous investigations have found that free radical scavengers (i.e., antioxidants), such as aspirin, mannitol, or genistein, can effectively reduce the oxidative DNA damage caused by free radicals [5]. These studies indicated that the inhibition of ROS-induced oxidative DNA damage is dependant on the concentration of radical scavengers. In light of this, we hypothesized that the presence of free radical scavengers will reduce the frequency and severity of oxidative DNA damage caused by VL-irradiated CQ/DMT in a concentration-dependent manner. We tested this hypothesis through treating ΦX-174 RF I SC double-stranded plasmid DNA with VL-irradiated 1.0 mM CQ/2.0 mM DMT/0.015 mM Cu (II) in the absence and presence of 10.0, 5.0, 2.5, 1.0, and 0.5 mM antioxidant (glutathione, *N*-acetyl-L-cysteine (NAC), mannitol, vitamin E, and vitamin C).

2. Materials and methods

2.1. Materials

CQ, cupric sulfate (Cu (II)), *N*-acetyl-L-cysteine (NAC), ascorbic acid, D-mannitol, vitamin E, glutathione (reduced), and DMT, all of the highest purity, were purchased from Sigma Aldrich (St. Louis, MO). SC ΦX-174 RF I double-stranded DNA was purchased from New England Biolabs (Beverly, MA). Hanks' balanced salt solution (HBSS) was purchased from Gibco/Invitrogen Corporation (Carlsbad, CA). Stock solutions of CQ and DMT of volume 1 M were prepared in ethanol and made fresh daily. All antioxidant stock solutions, except for vitamin E, were prepared daily in HBSS. Vitamin E was prepared fresh daily in ethanol as a 320 mM stock solution. The pH of each antioxidant stock solution was adjusted to 7.3–7.4 prior to use. A pH of 7.3–7.4 corresponds with the physiological pH range of human capillary blood [10] and unstimulated whole saliva [11].

2.2. Detection of oxidative DNA strand breaks

DNA strand breaks were measured by the conversion of SC ΦX-174 RF I double-stranded DNA to open circular (OC) and linear (L) forms. OC DNA indicates a single strand break, while L DNA is indicative of a more severe double strand break. In brief, 0.2 μg of DNA was incubated in HBSS, 0.015 mM Cu (II), 2.0 mM DMT, 1.0 mM CQ, in the absence and presence of various concentrations (10.0, 5.0, 2.5, 1.0, and 0.5 mM) of glutathione, NAC, mannitol, vitamin E, or vitamin C, at a final volume of 24 μl. VL-irradiated 1.0 mM CQ/2.0 mM DMT with 0.015 mM Cu (II), in the absence of an ROS scavenger, was used as a positive control (CQ/DMT system) and SC ΦX-174 RF I double-stranded DNA incubated in VL-irradiated HBSS with 0.015 mM Cu (II) was our negative control. The samples were irradiated with a Coltolux®75 VL dental lamp (470 nm light output > 900 mW/cm^2/Coltene-Whaledent Inc., Cuyahoga Falls, OH) for 60 s at a distance of 5.0 mm and incubated in 1.5 ml microcentrifuge tubes for 30 min at 37 °C. Following incubation, the samples were placed on ice and loaded into a 1% agarose gel containing 40 mM Tris, 20 mM sodium acetate, and placed in a horizontal slab electrophoresis box in Tris/acetate gel buffer. After electrophoresis the gels were stained with 0.5 μg/ml ethidium bromide solution for 20 min, followed by a 30 min wash in water. The gels were then photographed under ultraviolet illumination using a Kodak electrophoresis and analysis documentation system 290.

2.3. Data analysis

Results were obtained in three to five independent experiments for each antioxidant treatment. Digital images were analyzed with NIH Image J software, where the percentage of DNA in each form was calculated by measuring the area under the peaks. The results were calculated by determining the percentage of OC, L, and SC DNA in each sample. Furthermore, % damaged DNA was calculated with the following equation:

$$\%\ \text{Damaged DNA} = \frac{\text{Open circular} + \text{Linear}}{\text{Open circular} + \text{Linear} + \text{Supercoiled}} \times 100. \quad (1)$$