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Genotoxic effects of camphorquinone and DMT on human oral and intestinal cells

Miriam Wessels[*,1], *Julia Rimkus*[1], *Gabriele Leyhausen*, *Joachim Volk*, *Werner Geurtsen*

Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

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ABSTRACT

Objective. Released components of oral biomaterials can leach into the oral cavity and may subsequently reach the gastrointestinal tract. Camphorquinone (CQ) is the most common used photoinitiator in resinous restorative materials and is often combined with the co-initiator *N*,*N*-dimethyl-*p*-toluidine (DMT). It has been shown that CQ exerts cytotoxic effects, at least partially due to the generation of reactive oxygen species (ROS). Objective of this study was to examine the cytotoxic and genotoxic potential of CQ in human oral keratinocytes (OKF6/TERT2) and immortalized epithelial colorectal adenocarcinoma cells (Caco-2). Furthermore, the effects of visible-light irradiation and the co-initiator DMT were investigated as well as the generation of ROS, the potential protective effect of glutathione (GSH) and a recovery period of CQ-treated Caco-2 cells.

Methods. The alkaline comet assay was used to determine DNA damage. Additionally, an enzyme modified comet assay was applied, which detects 7,8-dihydro-8-oxoguanine (8-oxoguanine), a reliable marker for oxidative stress.

Results. Our data revealed that high concentrations of CQ induced DNA lesions in OKF6/TERT2 cells. This DNA damage is at least partly caused by the generation of 8-oxoguanine. In addition, CQ and DMT increased ROS formation and induced DNA damage in Caco-2 cells. CQ-treatment resulted in generation of 8-oxoguanine. The antioxidant GSH efficiently prevented CQ-associated DNA damage. Furthermore, a recovery following CQ-treatment significantly reduced DNA damage.

Significance. We conclude that CQ-induced DNA damage is caused by oxidative stress in oral and intestinal cells. These lesions can be prevented and possibly repaired by GSH-treatment and recovery of cells after the photoinitiator is removed from cultures.

1. Introduction

High amounts of various organic compounds may leach from resinous restorative materials in the first days after application. Subsequently lower quantities of these components are continuously released into the oral cavity due to degradation or erosion over time [1,2]. Leached substances, which are diluted by saliva, will end up in the gastrointestinal tract [3]. To date, no data is available in dental/medical literature about interactions of resinous compounds with intestinal cells. Camphorquinone (CQ) is the most important

* *Corresponding author*. Tel.: +49 511 532 4862; fax: +49 511 532 4811.
E-mail address: wessels.miriam@mh-hannover.de (M. Wessels).
[1] These authors contributed equally to this work.

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photoinitiator in dental composites [4] which is always combined with a co-initiator, such as *N*,*N*-dimethyl-*p*-toluidine (DMT) [5–7]. It has been calculated that CQ-concentrations up to 14 mM can potentially leach from light-curing resinous filling material [8]. Low concentrations of isolated CQ as well as in combination with DMT cause cytotoxic effects in cell cultures [9] and generate reactive oxygen species (ROS). It was documented that irradiated CQ and other photoinitiators can significantly increase ROS concentrations in various human cell types, which is associated with an enhanced toxicity [10,11]. However, a marked increase of ROS was also caused by CQ without light irradiation [12,13]. Intracellularly, elevated levels of ROS may result in alterations or damage of lipids, proteins and nucleic acids, which finally will cause cellular dysfunction [14,15]. Cells respond to oxidative stress with redox-regulating molecules and antioxidants such as glutathione (GSH) and enzymes like glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase [16,17]. It was found that antioxidants like GSH, ascorbic acid, and *N*-acetyl-L-cysteine (NAC) reduce high ROS concentrations, which were generated by CQ and other initiators [18–21].

It has been documented that CQ is also genotoxic. CQ was mutagenic in the bacterial *umu* test, and genotoxic in the DNA synthesis inhibition test at concentrations of 5 to 20 mM [22]. Single and double strand breaks in plasmid DNA were observed [20,23] as well as the formation of micronuclei in chinese hamster ovarian (CHO) cells exposed to CQ [18]. Nomura et al. [24] investigated genotoxicity using a bacterial assay. However, their results were inconclusive due to cytotoxic effects. Recently, Volk et al. [13] showed genotoxicity associated with elevated ROS levels for CQ concentrations up to 2.5 mM in human gingival fibroblasts using the comet assay.

The complex mechanisms of the CQ-induced genotoxicity are not yet fully understood. Therefore, it was objective of our study to analyze the effects of irradiated and non-irradiated CQ on human oral keratinocytes (OKF6/TERT2), which are one of the first target cells of released resinous substances, and immortalized epithelial colorectal adenocarcinoma cells (Caco-2). Goal of our experiments was to find out, whether CQ causes DNA strand breaks and oxidative DNA modifications due to elevated ROS levels in keratinocytes [25]. The hypothesis, which we set forth, was that non-irradiated CQ without and with DMT significantly elevate ROS levels and is genotoxic in cultures of immortalized human adenocarcinoma cells (Caco-2). Furthermore, we hypothesized that antioxidant GSH will prevent or reduce these effects.

The alkaline comet assay was used to determine DNA strand breaks and alkali labile sites. This method prove DNA damage, detecting single- and double-strand breaks, cross-links, incomplete excision repair sites as well as apurinic or apyrimidinic sites, which are alkali labile and therefore appear as breaks under the alkaline conditions of the assay [26]. The hOGG1-modified comet assay, which reveals oxidative DNA modifications and is an indicator of oxidative stress [27], was applied to analyze the formation of oxidized 7,8-dihydro-8-oxoguanine (8-oxoguanine) [28].

2. Materials and methods

2.1. Cell cultures

The immortalized human oral keratinocyte cell line OKF6/TERT2 was provided by Dr. J. Rheinwald (Harvard University). The keratinocytes were immortalized by transfection to express hTERT, the telomerase catalytic subunit, yet retain normal growth and differentiation characteristics [29]. OKF6/TERT2 cells were cultured in accordance with the protocols described by Dickson et al. [29] in a keratinocyte serum-free medium (ker-sfm no. 17005-042) containing 25 μg/mL bovine pituitary extract (BPE), 0.2 ng/mL epidermal growth factor (EGF) (all from GIBCO/Invitrogen, Darmstadt, Germany), 0.4 mM $CaCl_2$, and penicillin (100 U/mL)/streptomycin (100 mg/mL) (all from Biochrom KG, Berlin, Germany). For passaging, a 0.125% trypsin/0.01% EDTA solution (Sigma, Deisenhofen, Germany) in PBS and Dulbecco's modified Eagle medium/F-12 medium (DMEM/F-12, Biochrom KG, Berlin, Germany) including 10% fetal bovine serum (FBS, Lonza, Verviers, Belgium) was used. Exponentially growing cultures (5–8 d old) were used as source of cells for the next passage. For both experimental and control groups, cells were grown in a medium containing higher concentrations of nutrients (DF-K medium), mixed (1:1) of GIBCO ker-sfm and a DMEM/F-12 medium containing calcium-free, glutamine-free DMEM (#21068-028) with Ham's F-12 supplemented with 0.2 ng/mL EGF, 25 μg/mL BPE (#11765-054, all GIBCO/Invitrogen, Darmstadt, Germany), 1.5 mM/L glutamine, 2.5 μg/mL amphotericin and penicillin (100 U/mL)/streptomycin (100 mg/mL) (all from Biochrom KG, Berlin, Germany).

Caco-2 cells (clone C2BBe1), provided by Prof. Dr. U. Seidler (Hannover Medical School), were cultured in accordance with the protocols obtained from ATCC (American Type Culture Collection, Rockville, MD, USA). The colon carcinoma cell line was used as an in vitro model of the intestinal epithelium. For the experiments Caco-2 cells were cultured in Advanced MEM (Gibco BRL, Life Technologies, Eggenstein, Germany) containing 2 mM/L glutamine, 2.5 μg/mL amphotericin, penicillin (100 U/mL)/streptomycin (100 mg/mL), supplemented with 10% FBS. Cells were passaged by a short treatment with 0.25% trypsin/0.02% EDTA at regular intervals.

Both cell lines were maintained as monolayer cultures at 37 °C in a humidified atmosphere of 5% CO_2. Cell viability (95–98%) was estimated by using trypan blue dye and the TC10 automated cell counter before plating for experiments (all Bio-Rad Laboratories, Hercules, CA, USA). All cultures were routinely tested for mycoplasma contamination by means of the mycoplasma detection kit Venor GeM (Minerva Biolabs, Berlin, Germany).

2.2. Treatment of cells with dental materials and the antioxidant GSH

OKF6/TERT2 cells were pre-cultured for 24 h followed by a treatment with different CQ concentrations (2.5–5 mM) in the dark for 6 h. For visible light (VL-) activation of CQ, cells were