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Genotoxic and mutagenic potential of camphorquinone in L5178/TK$^{+/-}$ mouse lymphoma cells

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ABSTRACT

Objectives. Camphorquinone (CQ) is the most important photoinitiator used in dental composite resins. Sparse data indicate a mutagenic potential of CQ. Therefore, it was aim of this study to evaluate the cytotoxicity, genotoxicity, and mutagenicity of CQ in L5178Y TK$^{+/-}$ mouse lymphoma cells.

Methods. L5178Y/TK$^{+/-}$ cells were exposed to different concentrations of non-irradiated CQ (0.25–2.5 mM). Cytotoxicity was evaluated by propidium iodide assay, determination of suspension growth rate, relative total growth and the mitotic index. Intracellular levels of reactive oxygen/nitrogen species (ROS/RNS) were quantified by 2′,7′-dichlorofluoresceine diacetate (DCFH-DA). Early induction of DNA strand breaks and oxidative DNA base lesions was assessed using the 8-hydroxyguanine DNA-glycosylase 1 (hOGG1)-modified alkaline comet assay, whereas mutagenicity of CQ was determined in the mouse lymphoma TK assay (MLA), according to OECD Guideline No. 490.

Results. CQ (0.5–2.5 mM) induced concentration- and time-dependent inhibition of cell growth associated with increased ROS/RNS production, amounting to 2342% ± 1108% of controls after 90 min at 2.5 mM. Additionally, CQ concentration-dependently caused direct DNA-damage, i.e. formation of DNA strand breaks and 8-hydroxy-2′-deoxyguanosine. Whereas the MLA indicated lack of mutagenicity of CQ after a 4 h of treatment, CQ concentration-dependently increased total mutant frequency (MF) after 24 h (about 2-fold at 2.5 mM). But, based on the global evaluation factor concept, increase in MF did not reach biologically relevance.

Significance. CQ induced concentration-dependent, cytotoxic and genotoxic effects in L5178Y/TK$^{+/-}$ cells, most likely due to oxidative stress, but without mediating obvious biological relevant mutagenicity.

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1. Introduction

Camphorquinone (CQ) is the main photoinitiator in visible-light curing dental resinous materials [1]. The amount of CQ in resin composites determines the level of monomer–polymer conversion. Higher CQ concentrations usually lead to a higher conversion rate [2,3]. The amount of CQ in composites varies in a broad range from 0.17% to 1.03% (w/w) [4]. Subsequent to polymerization, low quantities of CQ are continuously released into the aqueous environment of the oral cavity, due to degradation and/or erosion over time [5,6]. It may be concluded that, apart from matrix monomers, which are contained in resinous materials in high quantities, water-extractable initiators like CQ are also of significance for the biological activity and safety of resin-modified oral biomaterials. These components may be released into surrounding tissues, i.e. the human pulp or the oral cavity, and may then mediate diverse toxic effects. The CQ concentrations to which cells and tissues are actually exposed *in vivo* are still unknown. Theoretical calculations, based on various published data, indicated maximum CQ concentrations of potentially 3–14 mM, which could leach from polymerized resins into the oral cavity. Leached amounts seem to be dependent on the composition of the resinous material, the oral conditions and further determinants (for details please see Ref. [7]). Durner et al. [8], for example, showed that more CQ was eluted from experimental resin composites containing silver nanoparticles to reduce caries than from conventional resin composites.

CQ is a reducible di-ketone, which may be enzymatically metabolized by one and/or two electron reduction steps. Therefore, it potentially has a high capacity of generating reactive oxygen species (ROS), which can cause a wide range of cellular effects. Based on this concept, we have previously demonstrated that both with and without additional light irradiation/activation CQ mediates a rapid, significant increase in intracellular ROS/RNS in primary human pulp and gingival fibroblasts [7,9], as determined by DCFH-DA. Additionally, Atsumi et al. [10,11] reported that irradiated CQ generated high amounts of ROS in primary human oral fibroblasts and a human submandibular cancer cell line. ROS/RNS can thereby exert multiple adverse effects in cells. In particular, high quantities of ROS/RNS acutely formed after CQ exposure may be responsible for cytotoxic and genotoxic effects.

The results of previous studies indicate that CQ causes cell type-specific and concentration-dependent cytotoxicity at low millimolar concentrations [12,13]. Furthermore, cell cycle arrest was observed in human gingival and pulp fibroblasts [14,15] and ROS-related apoptosis was detected in human oral keratinocytes [16]. Inhibition of DNA synthesis in HeLa cells by CQ was, however, masked by marked cytotoxicity of the photo-initiator [17].

Pagoria et al. [18] demonstrated in a cell-free system that irradiated CQ causes DNA strand breaks in supercoiled plasmid DNA. Using different genotoxicity assays, induction of variable levels of DNA damage by CQ was also found in different mammalian cell systems [7,16,19–21]. Pagoria and Geurtsen [20] showed that CQ induced initial DNA lesions represented by apurinic/apyrimidinic sites in immortalized murine cementoblasts. In addition, Li et al. [21] investigated the genotoxicity of CQ in combination with the reducing agent *N*,*N*-dimethyl-*p*-toluidine (DMT) with or without visible light (VL) irradiation. The authors showed that CQ in combination with DMT independent of VL irradiation induced micronuclei in Chinese hamster ovary cells. We recently demonstrated in comet assays with gingival fibroblasts that exposure to CQ can lead to induction of DNA strand breaks and/or abasic sites, which seem to be ROS-dependent [7,16]. Altogether, there is increasing evidence that CQ may possess DNA damaging potential.

But, the question whether CQ-mediated direct DNA damage may result in enhanced mutation frequencies in exposed cells still needs to be answered. The mutagenic potential of CQ has been only scarcely investigated. Up to now, prokaryotic cells were most frequently used. CQ was found to be non-mutagenic in the *Salmonella typhimurium* strain TA100 and in a battery of different bacterial strains with or without metabolic activation [22]. Nomura et al. [23] concluded that CQ has questionable mutagenic activity based on a marked toxicity using dark mutants of marine luminous bacteria (*Vibrio fischeri* M169). In contrast, CQ was clearly mutagenic in the bacterial *umu*-test [19]. Although each of these prokaryotic test systems detected mutagenic effects, these data cannot definitely predict mutagenicity in diploid mammalian cells.

Therefore, the present study aimed at assessing the DNA-damaging and mutagenic potential of CQ and the role of oxidative stress in CQ-induced DNA damage, using the L5178Y/TK$^{+/-}$ mouse lymphoma cell line. L5178Y/TK$^{+/-}$ cells are a highly sensitive, regulatory accepted *in vitro* model system for the analysis of somatic cell mutations, based on heterozygosity for the thymidine kinase (*TK*) gene. The respective so-called mouse lymphoma TK assay (MLA, [24]) is recommended by many regulatory agencies for determining mutagenicity of, e.g. chemicals, pharmaceuticals or materials coming into contact with food [25,26]. The assay uses the *TK* gene as reporter gene to detect both mutagenic (e.g. point mutations, small deletions, frame shift mutations) and clastogenic events (e.g. large deletions) and thus a wide range of genetic DNA alterations [27–29]. In the present study, the MLA was used in the presence and absence of an exogenous metabolizing system (S9-mix), i.e., a co-factor-supplemented post-mitochondrial fraction (S9) prepared from the livers of rodents to consider potential influence of metabolism on the (geno)toxic potential of CQ. Both CQ-induced DNA strand breaks/alkali labile sites and oxidative DNA base lesions were quantified by the very sensitive human 8-hydroxyguanine DNA-glycosylase 1 (hOGG1)-modified alkaline comet assay [30,31]. The hOGG1-modifed comet assay detects, in particular, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OH-dG, slides with hOGG1 incubation), as a major, premutagenic product of oxidative DNA attacks [32]. Since the molecular mechanisms of adverse biological activity of CQ may involve ROS generation, we furthermore monitored the levels of intracellularly generated ROS/RNS using the oxidation-sensitive dye 2,7-dichlorofluorescein diacetate (DCFH-DA).

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