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Assessment of cytotoxicity and DNA damage exhibited by siloranes and oxiranes in cultured mammalian cells

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Abstract

The potential reactivity and structural properties of oxiranes (epoxides) are advantageous when considering polymers for medical devices. However, epoxy compounds are widely known to have genotoxic properties. The objective of the study was to evaluate the cytotoxicity and primary DNA damage effects induced by oxiranes and siloranes, silicon containing oxiranes. The siloranes, Ph-Sil, Tet-Sil, and Sil-Mix and the oxiranes Cyracure™ UVR-6105 and 1,3-bis[2-(2-oxiranylmethyl) phenoxy]pentane (OMP-5) were dissolved in organic solvents and dilutions containing less than 0.5% solvent were used in biological assays. The concentration that reduced the viability of 50% (TC_{50}) of L929 cells was measured using the MTT assay and guided the selection of subtoxic doses for evaluation of DNA damage. Ph-Sil was more cytotoxic than OMP-5, Cyracure™ UVR-6105 and Sil-Mix. However, the TC_{50} value of Tet-Sil could not be determined due to its poor solubility. DNA damage was evaluated in the sister chromatid exchange (SCE) assay with CHO cells, and the alkaline comet assay with L929 cells. In contrast to the siloranes, the oxiranes exhibited significant increases ($p > 0.05$) in SCE frequencies and DNA migration relative to their solvent controls. Our findings support previous reports that siloranes have low genotoxic potential and can be suitable components for development of biomaterials.

Keywords: Sister chromatid exchanges; Comet assay; DNA damage; Cell culture; Siloranes; Silicon; Oxiranes

1. Introduction

Recently, researchers have reported the use of silicon-oxirane containing monomers (siloranes) in dental composites [1,2]. Silorane monomers need the potential reactivity of the epoxy group for polymerization. However, from past toxicological studies, epoxy groups in compounds are widely known to have genotoxic properties [3]. Interestingly, non-genotoxic oxirane monomers have been identified that have unique structural features such as spiroorthocarbonate [4,5] and silicon groups [6,7]. The mutagenicity of the siloranes Tet-Sil and Ph-Sil was reported much lower than related oxiranes in the Ames mutagenicity test [6] and mammalian cell mutagenicity tests [7]. Negative clastogenic effects on chromosomes were also reported in an in vitro mammalian cell micronucleus test [7]. The above tests measure effects on DNA as mutations or structural effects in the chromosome but do not measure primary DNA damage. The objective of this study was to evaluate primary DNA damage (genotoxicity) and non-specific cell damage (cytotoxicity) as a result of chemical effects

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of siloranes and oxiranes on cultured mammalian cells. Increases in SCE frequency and DNA migration were used as indicators of increased genotoxicity exhibited by the test compounds relative to controls. L929 mouse fibroblast (ATCC, CCL-1) cells were used in the comet assay as well as in cytotoxicity studies because of their mammalian genotypical and phenotypical similarities to mesenchymal oral tissues. However, for practical purposes, induction of SCE frequencies were obtained in Chinese hamster ovary (CHO) cells, a CHO cell has a short cell cycle (12–14 h) and a lower chromosome number ($n = 22$) than L929 cells ($n = 66$).

Cytogenetic methods have been used as biomarkers of exposure to DNA-damaging agents. The sister chromatid exchange (SCE) biomarker is considered to be a sensitive, rapid and simple cytogenetic end-point for evaluation of primary DNA damage [8]. A SCE takes place when a strand of DNA from one chromatid has exchanged with the corresponding strand of DNA of its sister chromatid. An increased frequency of SCEs can be correlated to greater DNA damage.

The comet assay or alkaline single cell gel electrophoresis (SCG) is another standard method for measuring DNA damage in single cells [9]. The assay is based on the embedding of cells in agarose, their lysis in alkaline buffer and afterwards subjection to an electrical current by electrophoresis. Cells embedded in agarose on a microscope slide are lysed to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix [10]. During electrophoresis, broken DNA fragments (damaged DNA) migrates away from the nucleus and become a tail behind the nucleus, resembling a comet tail, which is observed by fluorescence or silver nitrate staining. The alkaline (pH > 13) conditions of the assay enables detection of the broadest spectrum of DNA damage that can be the result of DNA single strand breaks, alkaline labile sites (primarily apurinic), single strand breaks associated with incomplete DNA excision repair sites, and DNA–DNA and DNA–protein cross-linking [9].

Evaluation of DNA damage in both assays requires the use of viable cells. Cell viability below 70% of that of control has been considered a sign of excessive cellular damage for proper interpretation of DNA damage [9,11]. Subtoxic concentrations can be determined using cell viability assays such as the methyl tetrazolium (MTT) assay [5] and Trypan blue exclusion assay [12].

2. Materials and methods

The chemical structures of the monomers examined in this study are shown in Fig. 1. The siloranes bis-3,4-epoxycyclohexylethyl-phenyl-methylsilane (Ph-Sil) and 3,4-epoxyclohexylethyl-cyclopolymethylsiloxane (Tet-Sil) and oxirane 1,3-bis[2-(2-oxiranylmethyl) phenoxy]pentane (OMP-5) were kindly donated by 3M ESPE company (Seefeld, Germany). The oxirane 3,4-epoxycyclohexylmethyl-3,4-epoxycyclohexylcarboxylate (Cyracure™ UVR-6105) was obtained from Union Carbide Corp., Danbury, CT. All monomers were viscous liquids of >95% purity, except Tet-Sil (>90%). A mixture of 50% Tet-Sil and 50% Ph-Sil by weight (Sil-Mix) was also evaluated in this study. The test chemicals were not water soluble with log P values >1.5. The highest dose tested in vitro was limited by a lack of solubility seen as excessive precipitation in the solvent/aqueous culture medium, in which the solvent did not exceed 0.5% (v/v).

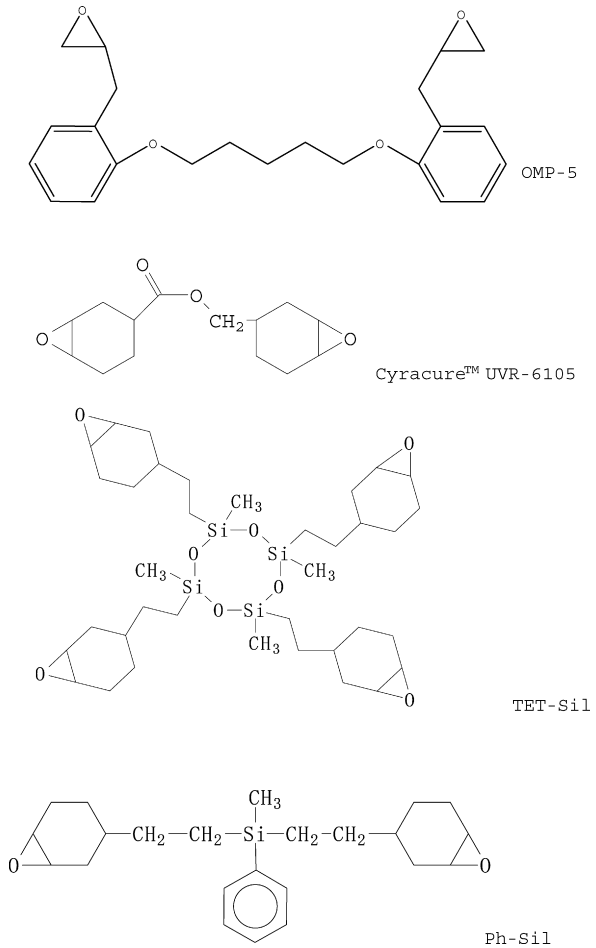

Fig. 1. Chemical structure of monomers.

2.1. Cell viability assay

Mouse fibroblast cells (American Type Culture Collection CCl 1 fibroblast NCTC clone L929) were grown in Eagle's minimal essential medium with Earle's balanced salt solution supplemented with 10% (v/v) fetal bovine serum, 2 mM L-