Mutation Research 815 (2017) 28–34

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Short communication

Cytotoxicities and genotoxicities of cements based on calcium silicate and of dental formocresol

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ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form 18 January 2017
Accepted 26 January 2017
Available online 20 February 2017

Keywords:
Cytotoxicity
Genotoxicity
Calcium silicate cement
Dental formocresol

ABSTRACT

Increasing interest is being paid to the toxicities of dental materials. The purpose of this study was to determine the cytotoxicities and genotoxicities of endodontic compounds to Chinese hamster ovary (CHO-K1) reproductive cells. Cultured CHO-K1 cells were treated with dental formocresol, two types of calcium hydroxide paste, and two types of mineral trioxide aggregate cement for 24 h. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay was performed on each culture, and the micronucleus frequency was determined by performing a micronucleus assay. Alkaline comet assay and γ-H2AX immunofluorescence assay were used to detect DNA damage. Out of the five materials tested, only dental formocresol significantly increased DNA damage. The mineral trioxide aggregate cements based on calcium silicate were not found to be potentially genotoxic. The data suggest that dental formocresol should not be recommended for use in vital pulp therapy on young teeth.

1. Introduction

Increasing interest is being paid to the toxicities of endodontic compounds. It is essential that dental pulp heals after traumatic injuries to immature permanent teeth or deciduous teeth to allow optimal recovery [1]. Partial pulpotomy or regenerative endodontic therapy can preserve vital pulp function in immature teeth that have been subjected to trauma and the pulp exposed. Maintaining dental pulp health after a traumatic injury is particularly important in immature permanent teeth because the completion of root formation requires pulp vitality to be retained. Dental formocresol and mineral trioxide aggregate (MTA) cement have similar pulpotomy success rates in primary molars [2], but dental formocresol may be cytotoxic, systemically distributed, and carcinogenic, meaning that less toxic alternatives are required [3]. Traumatized immature teeth are therefore often treated with calcium hydroxide [4] or, more recently, MTA cement [5]. Calcium hydroxide has been used in vital pulp therapy for a long time, but it gives poorer clinical and radiographic outcomes than do other materials [6]. In contrast, partial pulpotomy and regenerative endodontic therapy using MTA cement maintains pulp vitality and allows the physiological development of the tooth root to continue [7].

Endocem MTA cement, a new type of pozzolan cement, was recently developed by Maruchi (Wonju, Korea) and is marketed as a novel endodontic dental material [8]. It has been reported that Endocem MTA cement has a much shorter setting time and is more resistant to washout than ProRoot MTA cement (Dentsply, Tulsa, OK, USA) [9]. Endocem MTA and ProRoot MTA cements have similar biocompatibilities and odontogenic effects [10]. It has been suggested that ProRoot MTA cement is more appropriate than Endocem MTA cement for osteoblast-like cells, but this has not been proven [11].

In vitro genotoxicity tests are used to detect materials that damage genetic material and lead to DNA breaks, mutations, chromosomal breaks, or impaired ability to repair DNA, which is an important indicator of carcinogenesis [12]. Damaged chromosomes and their fragments can lead to the formation of micronuclei during the cell cycle interphase. The micronucleus assay can be used to detect chromosomal mutations, clastogenicity, and aneugenicity [13]. The single-cell gel electrophoresis assay is also useful for investigating the genotoxicities of compounds used for endodontic treatments [14]. The phosphorylated histone H2AX (γ-H2AX) detection assay can be used to detect double-strand breaks induced by particular compounds [15].

To the best of our knowledge, the genotoxicities of new dental cements based on calcium silicate to reproductive cells have not previously been determined. The aim of this study was to use genotoxicity assays to evaluate the cytotoxicities and genotoxicities of dental formocresol, two pastes based on calcium hydroxide

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http://dx.doi.org/10.1016/j.mrgentox.2017.01.001
1383-5718/

(Vitapex and Calcipex II), ProRoot MTA cement, and Endocem MTA cement to Chinese hamster ovary (CHO-K1) cells.

2. Materials and methods

2.1. Cell culture and reagents

The CHO-K1 cells used in the study were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in RPMI-1640 medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin–streptomycin (Gibco) at 37 °C in a 5% CO_2 atmosphere.

The test materials are listed in Table 1. Each test material was used at concentrations of 1, 10, and 100 μg/mL. These concentrations were selected from the results of previous genotoxicity tests [12,13] and preliminary experiments. Negative control group samples were treated with dimethyl sulfoxide (Sigma, St. Louis, MO, USA), and positive control group samples were treated with 1 μg/mL methyl meta-sulfonate (MMS; Sigma).

2.2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay

Each well of a 96-well plate was seeded with 1×10^5 cells/mL, then the plate was incubated at 37 °C for 24 h. A 100 μL aliquot of a test material (10 μg/mL) was then added to each cell, and the plate was incubated for 24 h. The contents of each cell were then subjected to a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay using an MTT assay kit (Trevigen, Gaithersburg, MD, USA) following the instructions provided by the manufacturer. Colorimetric changes at a wavelength of 570 nm were quantified using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.3. In vitro cytokinesis-block micronucleus assay

In vitro cytokinesis-block micronucleus (CBMN) assays were performed following current OECD guidelines [16,17]. Samples of cytochalasin B (4 μg/mL) were used as positive controls with and without S9 metabolic activation each time a test was performed. The positive control results are not shown later.

Cultured cells were exposed to 1, 10, or 100 μg/mL of a test material with and without cytochalasin B (4 μg/mL) present. The cytochalasin B was added in dimethyl sulfoxide (0.1 mL), then the cells were cultured for 24 h. The cultures were then incubated at 37 °C for 3 h (with or without S9 metabolic activation) or 24 h (without S9 metabolic activation). The 3 h cultures were then centrifuged for 10 min, washed twice with sterile saline, then resuspended in fresh medium containing fetal calf serum, gentamycin, and cytochalasin B (4 μg/mL). The cultures were then allowed to recover for 21 h at 37 °C.

After the cells had been treated, the test chemical was removed and the cells were harvested by adding 0.05% Trypsin EDTA. The cells were then resuspended in 0.075 mol/L KCl and incubated at 37 °C for 10 min. The cells were then fixed in a mixture of methanol and acetic acid for 10 min at room temperature, and this step was repeated two more times. Slides were prepared, and they were stained with 5% Giemsa solution for 10 min, then analyzed using an optical microscope at 400× magnification. The micronucleus frequency was defined as the number of binucleated cells (i.e., cells with micronuclei) per 1000 cells.

2.4. Single-cell gel electrophoresis assay (comet assay)

The single cell gel electrophoresis (comet) assay protocol met the guidelines proposed by Tice [18]. Cultured cells were incubated for 24 h, then exposed to 1, 10, or 100 μg/mL of a test material for 3 h in the absence or presence of an S9 mix. The cells were then subjected to an alkaline comet assay using a comet assay kit (Trevigen). The cells from a test were mixed with molten low melting-point agarose (1%) at a ratio of 1:10 (v/v), then the mixture was spread evenly over a CometSlide™ (Trevigen) and allowed to set for 30 min. The slide was then immersed in a lysis solution (2.5 mol/L NaCl, 0.1 mol/L EDTA, 10 mmol/L Tris base, 1% sodium lauryl sarcosinate, and 1% Triton X-100) for 30 min. Electrophoresis buffer (500 mmol/L EDTA and 200 mmol/L NaOH) was added, then electrophoresis at 21 V and 300 mA was conducted using a comet assay electrophoresis tank (Trevigen) for 30 min. The slide was then stained with SYBR green I for 5 min, then inspected using a fluorescence microscope (Carl Zeiss, Jena, Germany). The percentage

Table 1
Overview of the experimental compounds tested in this study.

Compound	Main composition	Type	Manufacturer
Dental Formocresol	40% formalin 40% cresol 20% ethanol	Liquid	Murakami, Shizuoka, Japan
Vitapex	30% $Ca(OH)_2$ 40.4% iodoform 22.4% silicone oil 6.9% inert	Paste	Neo Dental International Inc. Tokyo, Japan
Calcipex II	$Ca(OH)_2$ Barium sulfate Purified water Other	Paste	Nippon Shika Yakuhin Co, Shimonoseki, Japan
ProRoot MTA	55% C_3S 19% C_2S 10% C_3A 7% C_4AF 2.8% MgO 2.9% SO_3	Powder Liquid	Dentsply, Tulsa, OK, USA
Endocem MTA	46.7% CaO 5.4% Al_2O_3 12.8% SiO_2 3.0% MgO, 2.3% Fe_2O_3 2.4% SO_3 11.0% Bi_2O_3	Powder Liquid	Maruchi, Wonju-si,South Korea