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Transdentinal cytotoxicity of glutaraldehyde on odontoblast-like cells

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ABSTRACT

Objectives: This study investigated the transdentinal cytotoxicity of glutahaldehyde-containing solutions/materials on odontoblast-like cells.

Methods: Dentin discs were adapted to artificial pulp chambers. MDPC-23 cells were seeded on the pulpal side of the discs and the occlusal surface was treated with the following solutions: water, 2% glutaraldehyde (GA), 5% GA, 10% GA, Gluma Comfort Bond + Desensitizer (GCB + De) or Gluma Desensitizer (GDe). Cell viability and morphology were assessed by the Alamar Blue assay and SEM. The eluates were collected and applied on cells seeded in 24-well plates. After 7 or 14 days the total protein (TP) production, alkaline phosphatase activity (ALP) and deposition of mineralized nodules (MN) were evaluated.

Results: Data were analyzed by Kruskal-Wallis and Mann-Whitney tests ($p < 0.05$). GA solutions were not cytotoxic against MDPC-23. GCB + De (85.1%) and GDe (77.2%) reduced cell viability as well as TP production and ALP activity at both periods. After 14 days, GCB + De and GDe groups produced less MN. Affected MDPC-23 presented deformation of the cytoskeleton and reduction of cellular projections.

Conclusions: The treatment with 2.5%, 5% and 10% GA was not harmful to odontoblast-like cells. Conversely, when GA was combined with other components like HEMA, the final material became cytotoxic.

Clinical significance: Glutaraldehyde has been used to decrease dentin hypersensitivity. This substance is also capable of preventing resin-dentin bond degradation by cross-linking collagen and MMPs. This study showed that GA might be safe when applied on acid etched dentin. However, when combined with HEMA the product becomes cytotoxic.

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1. Introduction

Dentin hypersensitivity is a multifactorial disorder that results in an acute response to a non-noxious sensory stimulus.[1] Its primary cause appears to be the exposure of dentinal tubules in the oral cavity, which allows movement of dentinal fluid and generates a heightened response to tactile, chemical, thermal and osmotic stimuli that can range from mild discomfort to extreme pain.[2] A wide variety of treatments for dentin hypersensitivity is available. Topical application of products able to desensitize the nerve fibers or to occlude the dentinal tubules is the most common form of treatment.[3–5]

Glutaraldehyde (GA) is a cross-linking fixative and disinfecting agent that reacts with the ε-amino groups to induce the formation of cross-links.[6,7] In medical research GA is used in many different ways such as the construction of bioprosthetic heart valves,[8,9] modification of gelatins and other materials and tissues.[8,10,11] In dental field, it has been used to desensitize sensitive exposed dentin,[12] to inhibit MMPs[13] and to increase the mechanical properties of demineralized collagen prior to bonding procedures.[14]

Gluma Desensitizer® and Gluma Comfort® Bond + Desensitizer (De) contain 5% glutaraldehyde, 35% hydroxyethylmethacrylate (HEMA) and 60% water. GA is responsible for precipitating plasma proteins (especially albumin) in dentin to block dentin tubules[15] while HEMA reacts with this precipitate to form a mixture of polyHEMA and glutaraldehyde-cross-linked albumin[16] that reduces the movement of dentinal fluid and dentin hypersensitivity.

It has been shown that a solution containing only 5% GA applied for 60 s on 0.4-mm-thick dentin discs after acid-etching did not exert harmful effects on MDPC-23 cells.[17] Conversely, HEMA has been proven able to diffuse through dentinal tubules due to its hydrophylicity and small molecular weight.[18] Once this monomer reaches the pulp tissue it inhibits cellular metabolism and pulp tissue inherent defense mechanisms.[19–22] Taking into account that Gluma Dessensitizer and Gluma Comfort Bond + De contain both GA and HEMA in their composition the aim of this study was to evaluate the transdentinal cytotoxicity of these products and three different concentrations of GA on odontoblast-like cells.

2. Materials and methods

2.1. Preparation of dentin discs

Thirty-six sound third molars were obtained upon approval by the Ethics Committee of the Araraquara School of Dentistry—UNESP, and stored in 0.12% thymol solution at 4 °C for up to 3 months. One 0.5-mm-thick dentin disc with no enamel islets or pulp horn projections was obtained from the mid-coronal dentin of each tooth using a precision cutting machine equipped with a water-cooled diamond saw (Isomet 1000, Buehler Ltda., Lake Bluff, IL, USA). The occlusal side of the discs was then manually abraded with wet 320-grit silicon carbide paper to reach a final thickness of 0.4 mm as measured with a digital calliper providing a precision to 0.01 mm (Mitutoyo Sul Americana Ltda, Suzano, Sao Paulo, Brazil).

2.2. Permeability allocations

Dentin permeability was determined to permit a homogeneous distribution of the dentin discs into six groups ($n = 6$). The smear layer on both sides of the discs was removed by 0.5 M EDTA (pH 7.4) applied for 60 s. The discs were rinsed and individually placed in *in vitro* pulp chambers (IVPCs) modified from Hanks et al.[23] The IVPC was connected to a 180 cm column of water for 5 min, and after that the movement of a microbubble introduced through a metallic cannula was recorded during 1 min. The obtained values were transformed into hydraulic conductance values and the discs were allocated into the groups in such a way that the mean hydraulic conductance was statistically similar among them (ANOVA, $p > 0.05$). After measuring the permeability, a fresh smear layer was created on the occlusal side of each disc with a 600-grit silicon carbide paper for 10 s. Then, the discs mounted in the IVPCs were sterilized in ethylene oxide. An area of 0.28 cm^2 of exposed dentin was standardized for all discs by o'rings.

2.3. Seeding MDPC-23

MDPC-23 is an immortalized cell line from fetal mouse molar dental papillae able to express dentin sialoprotein and other proteins expressed by odontoblasts.[24,25] The cells were subcultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma Aldrich Corp., St. Louis, MO, USA) containing 10% fetal bovine serum (FBS; Cultilab, Campinas, SP, Brazil), 100 IU/mL penicillin, 100 μg/mL streptomycin and 2 mmol/L glutamine (Gibco, Grand Island, NY, USA) in an humidified incubator with 5% CO_2 and 95% air at 37 °C (Isotemp Fisher Scientific, Pittsburgh, PA, USA) for 3 days until reaching the number of cells necessary to perform the study. The cells (3×10^4) were seeded on the pulpal side of the dentin discs (0.28 cm^2) in 24-well plates (COSTAR 3595—Corning Incorporated, Corning, NY, USA) and maintained in an incubator with 5% CO_2 and 95% air at 37 °C. After 48 h the IVPCs were carefully removed from the compartments and returned to the same well with the occlusal side up to receive the treatment solutions/materials.

2.4. Application of the treatments

Six treatments were tested ($n = 6$) in this study: deionized water (control), 2.5% GA (Sigma-Aldrich Corp) in water, 5% GA in water, 10% GA in water, Gluma Dessensitizer and Gluma Comfort Bond + De (Heraeus Kulzer Inc., Armonk, NY, USA). The occlusal surface of the dentin discs was etched with 35% phosphoric acid (Scotchbond etchant, 3 M ESPE. St. Paul, MN, USA) for 15 s, carefully rinsed with deionized water for 10 s and blot dried with sterilized cotton pellets. Then, 20 μL of the GA solutions (2.5%, 5% or 10%) were applied for 60 s, followed by water rinsing and blot drying. Gluma Dessensitizer, Gluma Comfort Bond + D were applied according to the manufacturer instructions (Table 1). All procedures were performed in a vertical laminar flow chamber to prevent contamination and, immediately after, the IVPCs were placed again in a CO_2 incubator for additional 24 h.