Genotoxicity of corrosion eluates obtained from orthodontic brackets in vitro

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Introduction: The purpose of this study was to evaluate whether corrosion eluates obtained from commercially available orthodontic brackets are able to induce genetic damage in vitro. **Material and Methods:** Genotoxicity was assessed by the single cell gel (comet) assay using Chinese hamster ovary (CHO) cells. The following orthodontic metallic brackets were used: Morelli (Sorocaba, Brazil); Abzil (São José do Rio Preto, Brazil); Dentaurum (Pforzheim, Germany); and 3M Unitek (Puchheim, Germany). Each dental bracket was submitted to a corrosion process in a solution containing equal amounts of acetic acid and sodium chloride at 0.1 M concentration for 1, 3, 7, 14, 21, 35, and 70 days. CHO cells were exposed to eluates for 30 minutes at 37°C. The negative control was treated with the same solution used for corrosion process for 30 minutes at 37°C. Independent positive control was performed with methyl methanesulfonate (MMS) (Sigma Aldrich, St. Louis, Mo) at 1 ug/mL for 1 hour. **Results:** None of the eluates was found to exhibit genotoxicity, regardless of the different commercial brands of orthodontic appliance used. **Conclusions:** In summary, our results indicate corrosion eluates obtained from orthodontic brackets do not induce genetic damage as assessed by single cell gel (comet) assay. (Am J Orthod Dentofacial Orthop 2011;139:504-9)

Biocompatibility is the ability of a material to perform an appropriate host response in a specific application.[1] This means that the tissue of the patient that comes into contact with the material does not suffer from any toxic, irritating, inflammatory, allergic, genotoxic, or carcinogenic action.[2]

Accumulating evidence suggests that the oral environment is suitable for the biodegradation of metals as a result of its thermal, microbiologic, and enzymatic properties.[3] Intraoral fixed orthodontic appliances include brackets, bands, and arch wires that are made of alloys containing nickel, cobalt, and chromium in different percentages. Actually, different types of orthodontic brackets are available in the global market. The number of bracket systems for orthodontic therapy has increased significantly. Although they possess undeniable efficiency and local biocompatibility, there is concern about metal release from orthodontic devices to adjacent tissues such as oral mucosa cells and/or periodontal tissues. Thus, further biocompatibility data are needed in order to evaluate all risks of these components. Indeed, the limited data existing on the biocompatibility of these compounds appear to be insufficient.

Genotoxicity tests can be defined as in vitro and in vivo tests designed to detect compounds that induce genetic damage, including DNA damage, gene mutation, chromosomal breakage, altered DNA repair capacity, and cellular transformation. In the last decades, genotoxicity assays have gained widespread acceptance as an important and useful indicator of carcinogenicity.[4] As the incidence of head and neck cancer has increased in recent years—particularly in developing countries such as India, Vietnam, and Brazil, where it constitutes up to 25% of all types of cancer— risk factors other than tobacco smoke and the abuse of alcohol are of special concern.[5] Particularly, little information is available on the genotoxicity of orthodontic brackets so far.

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As a result of inappropriate evidence, the aim of this study was to evaluate whether corrosion eluates obtained from commercially available orthodontic brackets are able to induce genetic damage in vitro in order to predict the real risks when the corrosion process occurs during orthodontic therapy in vivo.

MATERIAL AND METHODS

Cell culture

In vitro test systems for genotoxicity evaluation can be differentiated into prokaryotic and/or eukaryotic tests. Studies conducted with the eukaryotic systems are thought to provide more reliable information with respect to the genotoxicity of chemicals.[6] Therefore, we aimed to investigate the genotoxic potential of corrosion eluates obtained from orthodontic brackets. For this we were able to use Chinese hamster ovary (CHO) cells. Our choice of CHO cells (a continuous cell line) provided an accurate evaluation of the changes independent from confounding factors such as age and metabolic and hormonal states of the donor. This cell line has a small number of relatively large chromosomes; they grow fast and reproducible results can be obtained from the same cell source.[7]

For this purpose, CHO cells (lineage CHO K-1) were growth to confluence in 75-cm^2 culture flasks (Corning, Incorporated, New York, NY) using Ham's F-10 medium (Invitrogen Corporation, Grand Island, NY) supplemented with 10% fetal calf serum and 100 U/mL penicillin (Life Technologies, Carlsbad, Calif) and 100 μg/mL streptomycin (Invitrogen Corporation) at 37°C with 5% CO_2. Cells were cultured for 5 days prior to treatment with test substances. Confluent cells were detached with 0.15% trypsin (Invitrogen Corporation) for 5 minutes; after that, 2 mL complete medium was added and cells were centrifuged at 1000 rpm (180 *g*) for 5 minutes. Cell suspension was counted using a Neubauer chamber and seeded in 96-well microtiter plates (Corning) at a density of 1×10^4 cells per well (at a concentration of 1×10^6/mL).

Cell treatment

For this study, the following commercially available orthodontic brackets were used: Morelli, Abzil, Dentaurum, and 3M Unitek. There are no differences regarding metal composition among them according to the manufacturer's instructions. Each dental bracket was submitted to a corrosion process in a solution containing equal amounts of acetic acid and sodium chloride (Merck & Co., Inc., Whitehouse Station, NJ), at 0.1 M concentration, for 1, 3, 7, 14, 21, 35, and 70 days. A volume of 10 uL of cells (approximately 10,000 cells) was then added individually to each final solution of eluate maintained for 30 minutes at 37°C. After exposure, cells were washed in phosphate buffered solution (PBS). The negative control was treated with the same solution used for the corrosion process for 30 minutes at 37°C. As cytotoxicity is a confounding factor in genotoxicity studies, it is not recommended to perform the single cell gel (comet) assay on samples with more than 30% cytotoxicity.[8] Thus, the exposure period as well as the final concentrations used here were established in a previous study conducted in our laboratory for evaluating the genotoxicity of these compounds only.[9] An independent positive control was performed with MMS at 1 μg/mL for 1 hour in order to ensure the reproducibility and sensitivity of the assay. In addition, each treatment was performed consecutively 3 times to ensure reproducibility.

Single cell gel (comet) assay

To evaluate the magnitude of DNA damage, we used the alkaline version of the single cell gel (comet) assay. The comet assay is a relatively new, rapid, simple, and reliable biochemical technique for evaluating DNA damage in mammalian cells.[8] This technique includes embedding cells in agarose gel on microscope slides, incubating them with the test compound, and then lysing the cells with detergent and high salts.[10] During electrophoresis under alkaline conditions, cells with damaged DNA display increased rates of DNA migration to the anode. The increase in DNA migration rate results from the formation of smaller fragments of DNA caused by double-strand breaks, single-strand breaks, and alkali-labile sites. Smaller fragments of DNA migrate further in the electric field compared with intact DNA, and the cellular lysates thus resemble a "comet" with brightly fluorescent head and a tail region. Our own recent studies have demonstrated that the single cell gel (comet) assay is a suitable tool to investigate genotoxicity of dental compounds used in clinical practice.[11-14]

The protocol used for the single cell gel (comet) assay followed the guidelines purposed by Tice et al.[8] Slides were prepared in duplicate per treatment. Thus, a volume of 10 μL of treated or control cells ($\sim 1 \times 10^4$ cells) was added to 120 μL of 0.5% low-melting-point agarose at 37°C, layered onto a precoated slide with 1.5% regular agarose, and covered with a coverslip. After brief agarose solidification in a refrigerator, the coverslip was removed and the slides were immersed in the lysis solution (2.5 M NaCl, 100 mM ethylenediaminetetraacetic acid [EDTA] [Merck & Co., Inc.]; 10 mM Tris-HCl buffer pH = 10 [Sigma Aldrich, St. Louis, Mo]; 1% sodium sarcosinate [Sigma Aldrich]; with 1% Triton X-100 [Sigma Aldrich]; and 10% DMSO [Merck & Co. Inc.]) for about 1 hour. Prior to electrophoresis, the slides were left in an alkaline