Genotoxicity of endodontic irrigants and medicaments

Abstract

Genotoxicity describes a deleterious action on a cell’s genetic material affecting its integrity. This includes both certainchemical compoundsand certain types ofradiation. Genotoxic substances are all those with affinity to interact with DNA – which is not proof of their dangerousness to humans, but does render them potentially mutagenic or carcinogenic. This review will address the genotoxicity of endodontic irrigants, medicaments, and sealers.

It has been postulated that exposure of living tissues to cytotoxic agents can result in chronic cell injury, compensatory cell proliferation, hyperplasia, irritation, degeneration or tissue necrosis (1) and ultimately tumor development (2, 3). It is likely that proliferation may increase the risk of mutations within target cells and also be important in selective clonal expansion of exogenously or endogenously initiated cells from pre-neoplastic foci and eventually tumors (2). Thus, the DNA damage may diminish the self-repairing potential of tissue (4). In light of these considerations, genotoxicity and cytotoxicity assays gained widespread acceptance as an important and useful indicator of carcinogenicity.

Definition of genotoxicity

Ingenetics, genotoxicity describes a deleterious action on a cell’s genetic material affecting its integrity. This includes both certainchemical compoundsand certain types ofradiation. Genotoxic substances are all those with affinity to interact with DNA – which is not proof of their dangerousness to humans, but does render them potentially mutagenic or carcinogenic (5).

Genotoxicity of root canal irrigants

Sodium hypochlorite

Sodium hypochlorite (NaOCl) is recommended as the main endodontic irrigant because of its ability to dissolve organic matter together with its broad antimicrobial action (6). NaOCl is commercially available as aqueous solutions with concentrations ranging from 1% to 15% and having an alkaline pH with values around 11 (7).

Sodium hypochlorite has a wide range activity against both Gram positive and Gram negative bacteria. It is the strongest antifungal agent among root canal irrigations and medications. Furthermore, it is the only root canal irrigant that can destroy the microbial biofilm effectively (6, 7).

Hamaguchi and Tsyutsui (8) showed that NaOCl was not genotoxic to mamalian cells. Hagiwara et al. (9) showed that sodium hypochlorite induced chromosome aberrations in Syrian hamster embryo (SHE) cells when treated in the presence of exogenous metabolic activation. Aubut et al. (10) revealed that neutralizing a 2. 5% NaOCl solution did not induce any genotoxic effect. Marins et al. (11) showed that NaOCl did not induce any genotoxic effect.

Chlorhexidine

CHX is a synthetic cationic bis-guanide that consists of two symmetric 4-cholorophenyl rings and two biguanide groups connected by a central hexamethylene chain. CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of

active or passive transport mechanism (12). Its efficacy is due to the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls (12), thereby altering the cells' osmotic equilibrium. This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacteria. CHX is a base and is stable as a salt. The most common oral preparation, chlorhexidine gluconate, is water-soluble and at physiologic pH, it readily dissociates and releases the positively charged chlorhexidine component (12). At 0. 2% concentration, due to the leakage of low molecular weight substances(potassium and phosphorous) from the bacterial cell wall, CHX is bacteriostatic. On the other hand, at 2% concentration, CHX is bactericidal, as precipitation of the cytoplasmic contents occurs and resulting in cell death (13).

Ribeiro et al. (14) revealed that chlorhexidine digluconate is able to induce primary DNA damage in leukocytes and in oral mucosal cells, but no chromosome breakage or loss in erythrocytes. Another study indicated that CHX in 0. 01% and 1% concentrations did not induce DNA damage.

Yeung et al. (15) stated that potential genotoxicity and tissue damage when extruded into the periradicular tissue and at higher concentrations should be considered during periodontal and endodontic practice. Li et al. (16) revealed that CHX-induced genotoxicity on macrophages may be via reactive oxygen species generation.

MTAD

BioPure (Dentsply, Tulsa Dental, Tulsa, OK, USA), otherwise known as MTAD, was introduced by Torabinejad *et al.* in 2003 (17). It is composed of 3% doxycycline, 4. 25% citric acid and a detergent (0. 5% Polysorbate 80) (17).

Marins et al. (18) assessed the genotoxicity of MTAD using single cell gel (comet) assay. Results showed that the BioPure MTAD was able to promote DNA breakage in CHO cells only at the highest concentration tested as well as to induce significant increase in tail moment at all tested concentrations in murine fibroblasts. Another study revealed that MTAD did not cause cell death, but presented genotoxic effects (19).

EDTA

EDTA (Ethylenediaminetetraacetic acid) refers to the chelating agent with the formula (HO2CCH2)2NCH2CH2N(CH2CO2H)2. This amino acid is widely used to sequester di- and tri-valent metal ions. EDTA binds to metals via four carboxylate and two amine groups. EDTA forms specially strong complexes with Mn(II), Cu(II), Fe(III), and Co(III). EDTA is mostly synthesised from 1, 2-diaminoethane (ethylenediamine), formaldehyde (methanal), water

and sodium cyanide. This yields the tetra sodium salt, which can be converted into the acidic forms by acidification (20, 21).

EDTA is apolyamino carboxylic acidand a colourless, water-soluble solid. It is widely used to dissolvelimescale. Its usefulness arises due to its role as a hexadentateligandandchelating agent, i. e. its ability to sequestermetalionssuch as Ca2+ and Fe3+. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. EDTA is

produced as several salts, notably disodium EDTA and calcium disodium EDTA. The compound was first described in 1935 by Ferdinand Munz, who prepared the compound fromethylenediamineandchloroacetic acid(22). Today, EDTA is mainly synthesised fromethylenediamine(1, 2-diaminoethane), formaldehyde, andsodium cyanide(22).

EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. EDTA demineralizes dentine to a depth of 20–30 μm in 5 min (23).

According to Heindorff et al. (24) EDTA influences chromosome breakage by mutagenic agents. In particular, when applied in combination with chemical mutagens, EDTA enhances mutagen-induced aberration frequencies. Furthermore, they reported that EDTA affects the inhibition of DNA synthesis in primary cultures of mammalian cells. This may be due to impairment of enzymes involved in DNA replication. Using single cell gel (Comet)assay, Marins et al. (25) showed that EDTA did not produce genotoxic effects.

Iodine potassium iodide (IKI)

Iodine was first discovered in seaweed in the early 1800s. Although its exact mode of action is not fully known, it is thought to induce cell death nonspecifically due to the oxidizing effects of free iodine on SH-OH- and NH groups of amino acids and on double bonds of unsaturated fatty acids. Iodine is highly efficient microbicide to a wide variety of bacterial, fungal and viral infections (26).

Potassium iodide (KI) is a compound made of 76% of iodine and 23% of the alkali metal potassium by weight. KI is prepared by reacting iodine with a hot

solution of potassium hydroxide, the product being subsequently reduced to iodide by heating the dry reaction mixture with carbon. Another form of iodine compounds is IKI. The solution can be prepared by mixing 2 g of iodine in 4 g of KI; this mixture then is dissolved in 94 ml of distilled water (26).

Poul et al. (27) assessed the genotoxic effects of potassium iodate in vitro using the alkaline comet assay and the cytokinesis-block micronucleus assay on CHO cells.

Results showed that potassium chlorate as well as potassium iodide, bromide and chloride did not induced DNA damage in the alkaline comet assay for doses up to 10 mM. In another study, Hikiba et al. (28) assessed the effect of iodine and iodoform on chromosome aberrations using Syrian hamster embryo (SHE) cells and found that iodine induced chromosome aberrations and iodoform induced no genotoxicity. Using the comet assay and chromosome aberration test to characterize the genotoxic potency of povidone-iodine within 4 h of contact with CHO-K1 cells, Muller et al. (29) found no chromosomal damage. In another study, Hedayati et al. (30) showed that incubation of lymphocytes with (131)I induced genotoxicity, which was reflected by an increase in micronuclei frequency.

Genotoxicity of intracanal medicaments

Calcium hydroxide

Calcium hydroxide [Ca(OH)2] was originally introduced to the field of endodontics by Herman in 1920 as a pulp-capping agent. It is a white

odorless powder with the formula Ca(OH)2, and a molecular weight of 74. 08 (31). Calcium hydroxide has low solubility in water (about 1. 2 gL-1 at 25 C), which decreases as the temperature rises. The dissociation coefficient of Ca(OH)2 (0. 17) permits a slow, controlled release of both calcium and hydroxyl ions. The low solubility is a good clinical characteristic as a long period is necessary before it becomes soluble in tissue fluids when in direct contact with vital tissues (32). It has a high pH (about 12. 5-12. 8), is insoluble in alcohol and is chemically classified as a strong base, it main actions come from the ionic dissociation of Ca2+ and OH- ions and their effect on vital tissues, generating the induction of hard tissue deposition and being antibacterial. Ca(OH)2 dissociates into calcium and hydroxyl ions on contact with aqueous fluids. Ca(OH)2 in water has a thixotropic behavior (33).

According to Ribeiro et al. (34) calcium hydroxide do not promote DNA damage in mammalian cells. In another study, Ribeiro et al. (35) revealed that calcium hydroxide was not able to modulate alkylation-induced genotoxicity or oxidative DNA damage as depicted by the single cell gel (comet) assay.

MTA

MTA is a mixture of a refined Portland cement and bismuth oxide and trace amounts of SiO2, CaO, MgO, K2SO4, and Na2SO4 (36). Portland cement is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, and tetracalcium aluminoferrite (37). Gypsum, and to a lesser extent, tetracalcium aluminoferrite are important determinant of setting time

(37). MTA contains fewer toxic heavy metals, has a longer working time, and have undergone additional processing/purification than regular Portland cements (36). The MTA powder is mixed with supplied sterile water in a 3: 1 powder/liquid ratio and it is recommended that a moist cotton pellet be temporarily placed in direct contact with the material and left until a follow-up appointment (38). Upon hydration, MTA materials form a colloidal gel that solidifies to a hard structure in approximately 3–4h, with moisture from the surrounding tissues purportedly assisting the setting reaction (38). Hydrated MTA has an initial pH of 10. 2, which rises to 12. 5 three hours after mixing (39).

Using single cell gel (comet) assay, Ribeiro et al. (40) detected no DNA damage after a treatment of cells by MTA and Portland cements for concentrations up to 1000 µ/ml. In another study, Ribeiro et al.(41) demonstrated that regular and white MTA did not produce genotoxic effects at 1 to 1000 µgmL-1 FOR 3 H AT 37 C. Another study using Chinese hamster ovary (CHO) cells indicated that that MTA and Portland cements are not genotoxins and are not able to induce cellular death (42). Braz et al. (43) assessed the genotoxic effects of MTA and Portland cements in peripheral lymphocytes from 10 volunteers by the alkaline single cell gel (comet) assay. Findings failed to detect the presence of DNA damage after a treatment of peripheral lymphocytes by MTA and Portland cements for concentrations up to 1000 mug mL(-1). Camargo et al. (44) revealed that regular and white MTA preparations did not negatively influence cell survival or reactive oxygen species production. Ding et al. (45) showed that MTA and calcium

silicate possessed no genotoxic effect. According to Zeferino et al. (46) MTA as well as Portland cement+15% bismuth oxide were not genotoxic.

Genotoxicity of root canal sealers

For assessment of the genotoxic potential of any material, it is recommended to perform a series of in vitro tests. At least two assays, investigating different endpoints, shall use mammalian cells. For cytotoxic and bactericidal compounds, as many endodontic sealers appear to be, care must be taken in the test set up: For a proper evaluation the selected test concentrations used for genotoxic effects must be below the concentrations where toxic effects are found (47).

Ørstavik and Hongslo (48) showed that extracts of a synthetic polymer material, based on epoxy-bis-phenol A, induced mutations in Salmonella typhimurium TA 100 as did extracts of the epoxy-bis-phenol A resin alone. Formaldehyde, an active ingredient from one of the ZnO-based materials, induced mutations in both Salmonella typhimurium TA 98 and TA 100. The mutagenic activity of formaldehyde as well as of the epoxy material was reduced in the presence of rat liver microsomes.

Schwikl et al. (49) showed that eluates of mixed AH26 were mutagenic, and their genotoxicity was strongly depended on the setting time. The number of mutants after exposure to eluates of unset AH26 was enhanced approximately 7- to 10-fold. However, the mutagenic activity of the mixed material was clearly reduced after a setting time of 1 wk. Physiological saline eluates of the mixed AH26 were not found to be mutagenic. Dimethyl sulfoxide eluates of the liquid component of AH26 elicited mutagenic effects

similar to the freshly mixed material; eluates made in physiological saline were barely mutagenic at a 10-fold higher concentration.

Leyhausen et al. (50) showed that AH-Plus revealed no genotoxicity and mutagenicity.

Epoxy-based sealers are also mutagenic in mammalian cell mutation assays. Ersev et al. (51) showed that silver-free AH26 set for 24 h were weakly mutagenic in Salmonella typhimurium TA100. They further showed that silver-free AH26 might contain small amounts of two mutagenic substances: bisphenol A diglycidyl ether and formaldehyde. Tai et al. (52) revealed that root canal sealers containing formaldehyde and bisphenol A diglyether proved to be not only cytotoxic but also genotoxic. Miletic et al. (53) found no mutagenicity found for AH26 and AH Plus sealers on human lymphocytes in highly controlled conditions in vitro.

Formaldehyde is released from some epoxy-based sealers with a maximum after 2 days, even though the amount is much less than that of paraformaldehyde containing zinc oxide-eugenol sealers (54). It was believed that the leakage of formaldehyde and bisphenol-

A diglycidyl ether from the epoxy-sealers contributed to the mutagenic effects (49, 55).

Formaldehyde is classified as a carcinogen in animals, whereas there exists only limited evidence for carcinogenic effects in man (56). There is also limited evidence for animal carcinogenicity from bisphenol-A diglycidyl ether and no adequate data for the evaluation of human cancer risk for this

compound (57). Considering the limited exposure of these compounds from endodontic epoxy sealers and the lack of definitive assessment by the IARC, it seems unlikely that such sealers contribute to an increased risk of cancer in patients. However, the high level of paraformaldehyde in zinc oxide-eugenol.

Using Comet assay, Huang et al. () showed that the zinc oxide eugenol-based sealers (Canals, Canals-N, and Tubilseal) did not always cause a dose-dependent increase in genotoxicity. The resin-based sealers (Topseal, AH 26, and AH Plus) caused a dose-dependent increase in genotoxicity, but no such effect was seen with the calcium hydroxide-based sealer (Sealapex) (47).