Genotoxicity of Endodontic Materials: A Critical Review

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ABSTRACT
Genotoxicity is an action on cell’s genetic material which may affect its integrity. This includes certain types of radiations and also certain chemical compounds. Genotoxic materials are those with affinity to interact with DNA but render them potentially carcinogenic or mutagenic. This review will address the genotoxicity of endodontic irrigants, medicaments and sealers.

Keywords: Endodontics, Genotoxicity, Sealer.


INTRODUCTION
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, and ultimately tumor development. 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 preneoplastic foci and eventually tumors. Thus, the DNA damage may diminish the self-repairing potential of tissue. In light of these considerations, genotoxicity and cytotoxicity assays worldwide acceptance as an important indicator of carcinogenicity.

DEfINITION Of GENOTOxICITy
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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. It is commercially available as aqueous solutions with 1 to 15% concentrations and having an alkaline pH (around 11).

Hamaguchi and Tsutsui showed that NaOCl was not genotoxic to mammalian cells. Hagiwara et al showed that NaOCl induced chromosome aberrations in Syrian hamster embryo cells. Aubut et al revealed that neutralizing a 2.5% NaOCl solution cannot induce genotoxic
effect. Marins et al showed that NaOCl induced no genotoxic effect.

**Chlorhexidine**

Chlorhexidine (CHX) is a synthetic cationic bisguanide that consists of two biguanide groups and two symmetric 4-chlorophenyl rings with the connection of hexamethylene chain. It is a positively charged molecule that interacts with lipopolysaccharides and phospholipids on bacterial cell membrane, resultantly entering the cell through some type of transport mechanisms. Its efficacy is the result of interaction of the negatively charged phosphate groups on cell walls and the positive charge on the molecule, thereby changing the osmotic equilibrium. This can increase the cell wall permeability, allowing the CHX to enter the microbial cell. Chlorhexidine gluconate, which is the most common oral product, readily dissociates and releases the positively charged CHX component at physiologic pH. 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 resulting in cell death.

Ribeiro et al showed that CHX digluconate can induce primary damage in DNA of leukocytes and mucosal cells, but no chromosome breakage in red blood cells. Another study indicated that CHX in 0.01 and 1% concentrations did not induce DNA damage.

Yeung et al stated that potential genotoxicity when extruded into the periapical tissues and at higher concentrations, must be considered during endodontic treatment. Li et al revealed that CHX-induced genotoxicity on macrophages may be via reactive oxygen species generation.

**BioPure MTAD**

BioPure (Dentsply, Tulsa Dental, Tulsa, OK, USA), otherwise known as MTAD, was introduced by Torabinejad et al in 2003. It is composed of 4.25% citric acid, 3% doxycycline and 0.5% polysorbate 80, which is a detergent.

Marins et al assessed the genotoxicity of MTAD using single cell gel (comet) assay. Results indicated that BioPure MTAD can promote DNA breakage only at the highest concentrations and also can induce significant increase in tail moment at all concentrations. Another study revealed that MTAD did not cause cell death, but presented genotoxic effects.

Ethylenediaminetetraacetic acid: Ethylenediaminetetraacetic acid (EDTA) refers to an amino acid which is widely used to sequester di- and trivalent metal ions. It binds to metals via 2-amino group and 4-carboxylate. It forms especially strong complexes with Cu, Fe, Mn and Co. It is mostly synthesized from 1, 2-diaminoethane, formaldehyde, water and sodium cyanide.

The EDTA is a water-soluble colorless solid which is used for dissolving lime scale. Its usefulness is due to its ability to sequester metal ions. The compound was initially described in 1935 by Munz, who prepared the compound from chloroacetic acid and ethylenediamine.

Ethylenediaminetetraacetic acid reacts with calcium ions in dentin and demineralizes dentin up to 20 to 30 μm depth in 5 minutes.

According to Heindorff et al, EDTA influences chromosome breakage, particularly when applied in combination with chemical mutagens. Also, it affects the inhibition of DNA synthesis of mammalian cells. Marins et al also showed that it produces no genotoxic effect.

**Iodine Potassium Iodide (IKI)**

Iodine was firstly discovered in seaweed. 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 a highly efficient microbicide to a wide variety of bacterial, fungal and viral infections.

Potassium iodide (KI) is prepared by a reaction between iodine and a hot solution of potassium hydroxide. Another form of iodine compounds is IKI. The solution can be prepared by mixing 2 gm of iodine in 4 gm of KI; this mixture is then dissolved in 94 ml of distilled water.

Poul et al assessed the genotoxic effects of KI in vitro on Chinese hamster ovary (CHO) cells and concluded that potassium chlorate and potassium iodide, chloride and bromide did not induce DNA damage for doses up to 10 mm. In another study, Hikiba et al 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, Muller et al found no chromosomal damage. In another study, Hedayati et al showed that incubation of lymphocytes with iodine increased micronuclei frequency and induced genotoxicity.

**GENOTOXICITY OF INTRACANAL MEDICAMENTS**

**Calcium Hydroxide**

Calcium hydroxide \([\text{Ca(OH)}_2]\) was originally introduced to the endodontics in 1920 as a pulp capping material. It is an odorless powder with molecular weight of 74.08.
Calcium hydroxide has low solubility in water, which decreases as the temperature increases. The low solubility of the material is a suitable clinical characteristic. It dissociates into hydroxyl and calcium ions on contact with fluids. In water, Ca(OH)$_2$ may show a thixotropic behavior.

According to Ribeiro et al, Ca(OH)$_2$ cannot promote DNA damage. In another study, Ribeiro et al revealed that it was not able to modulate genotoxicity or even oxidative DNA damage.

**Mineral Trioxide Aggregate (MTA)**

This material is a combination of refined Portland cement, bismuth oxide and some amounts of K$_2$SO$_4$, MgO, SiO$_2$, CaO, and Na$_2$SO$_4$. Portland cement is a combination of tricalcium silicate, dicalcium silicate, gypsum, tricalcium aluminate and tetracalcium aluminoferrite. It has a better working time and has undergone additional processing than regular portland cement. The powder should be mixed with sterile water in a 3:1 powder/liquid ratio. After hydration, MTA solidifies to a hard material in near 3 hours, by absorbing moisture from the surrounding tissues. Hydrated MTA shows initial pH of 10.2, which rises to 12.5 after 3 hours.

Using single cell gel (comet) assay, Ribeiro et al detected no DNA damage after a treatment of cells by MTA for up to 1000 μg/ml concentrations. In another study, Ribeiro et al demonstrated that regular and white MTA did not produce genotoxic effects at 1 to 1000 μg/ml for 3 hours at 37°C. Another study using CHO cells indicated that MTA is not genotoxic. Braz et al assessed the genotoxic effects of MTA in lymphocytes and failed to detect DNA damage. Camargo et al revealed that MTA cannot negatively influence cell survival. Ding et al showed that MTA and calcium silicate possessed no genotoxic effect. According to Zeferino et al, MTA as well as Portland cement and 15% bismuth oxide were not genotoxic.

**Genotoxicity of Root Canal Sealers**

For evaluation of the genotoxicity of any material, performance of some *in vitro* tests is recommended. For bactericidal and cytotoxic compounds, care must be taken in setting up of the test.

Ørstavik and Hongslo concluded that a synthetic polymer based on epoxy-bis-phenol A, induced mutations. Formaldehyde-induced mutations; its mutagenic activity was reduced in presence of liver microsomes.

Schwikl et al concluded that mixed AH26 was mutagenic, and the genotoxicity depended on setting time. Physiological saline eluates of mixed AH26 were not mutagenic.

Leyhausen et al showed that AH-plus revealed no genotoxicity and mutagenicity. Epoxy-based sealers have also been shown to be mutagenic. Ersev et al showed that silver-free AH26 set for 24 hours were weakly mutagenic. They further showed that silver-free AH26 may contain few amounts of two mutagenic materials (formaldehyde and bisphenol A diglycidyl ether). Tai et al revealed that sealers containing these two materials proved to be cytotoxic but and genotoxic. Miletic et al found no mutagenicity of AH26 and AH Plus sealers on human lymphocytes in highly controlled conditions *in vitro*.

Formaldehyde is released from some epoxy-based sealers (maximum rate after 48 hours), even though this amount is less than that of paraformaldehyde. The leakage of formaldehyde from the epoxy sealers has been contributed to the mutagenic effects. Formaldehyde is carcinogenic in animals; however, there are only few evidences for showing carcinogenicity in human. Considering the low exposure of these materials from epoxy sealers, it seems that such sealers do not contribute to increased risk of carcinogenicity in human.

**CONCLUSION**

Genotoxicity is an action on cell’s genetic material which may affect its integrity. Genotoxic materials are those with affinity to interact with DNA but render them potentially carcinogenic or mutagenic. Exposure of living tissues to cytotoxic agents can result in chronic cell injury, compensatory cell proliferation, hyperplasia, irritation, degeneration or tissue necrosis and ultimately tumor development. Thus, the DNA damage may diminish the self-repairing potential of tissue. Genotoxicity of materials used in endodontics such as canal irrigants, sealers, and medicaments should be assessed before their usage in conventional practice.

**REFERENCES**


