10.5005/jp-journals-10024-1742

REVIEW ARTICLE

Genotoxicity of Endodontic Materials: A Critical Review

[1]Zahed Mohammadi, [2]Sousan Shalavi, [3]Hamid Jafarzadeh, [4]Shilpa Bhandi, [5]Shankargouda Patil

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.

How to cite this article: Mohammadi Z, Shalavi S, Jafarzadeh H, Bhandi S, Patil S. Genotoxicity of Endodontic Materials: A Critical Review. J Contemp Dent Pract 2015;16(8):692-696.

Source of support: Nil

Conflict of interest: None

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.[1-3] 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 preneoplastic 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 worldwide acceptance as an important indicator of carcinogenicity.

[1]Iranian Center for Endodontic Research, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iranian National Elites Foundation, Tehran Iran

[2]Private Dental Practice, Hamedan, Iran

[3]Department of Endodontics, Dental Research Center Faculty of Dentistry, Mashhad University of Medical Sciences Mashhad, Iran

[4]Department of Conservative Dentistry and Endodontics Faculty of Dental Sciences, MS Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India

[5]Department of Oral and Maxillofacial Pathology, Faculty of Dental Sciences, MS Ramaiah University of Applied Sciences Bengaluru, Karnataka, India

Corresponding Author: Hamid Jafarzadeh, Associate Professor Department of Endodontics, Dental Research Center, Faculty of Dentistry, Mashhad University of Medical Sciences, Vakilabad Blvd, Mashhad, Iran, P.O. Box: 91735-984 Phone: +98-51-38829501, e-mail: hamid_j365@yahoo.com JafarzadehBH@mums.ac.ir

DEFINITION OF GENOTOXICITY

In genetics, genotoxicity is an action on cell's genetic material which may affect its integrity. This includes certain types of radiation and chemical compounds. Genotoxic materials are those with affinity to interact with DNA but render them potentially carcinogenic or mutagenic.[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] It is commercially available as aqueous solutions with 1 to 15% concentrations and having an alkaline pH (around 11).[7]

Sodium hypochlorite has wide activity against both gram negative and positive bacteria. It has the strongest antifungal activity among canal irrigants/medications. Furthermore, it is the only endodontic irrigant with the ability of destroying the microbial biofilm.[6,7]

Hamaguchi and Tsutsui showed that NaOCl was not genotoxic to mamalian cells.[8] Hagiwara et al showed that NaOCl induced chromosome aberrations in Syrian hamster embryo cells.[9] Aubut et al revealed that neutralizing a 2.5% NaOCl solution cannot induce genotoxic

effect.[10] Marins et al showed that NaOCl induced no genotoxic effect.[11]

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.[12] 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.[12] 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.[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 resulting in cell death.[13]

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.[14] 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.[15] Li et al revealed that CHX-induced genotoxicity on macrophages may be via reactive oxygen species generation.[16]

BioPure 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 4.25% citric acid, 3% doxycycline and 0.5% polysorbate 80, which is a detergent.[17]

Marins et al assessed the genotoxicity of MTAD using single cell gel (comet) assay.[18] 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.[11]

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-amine 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.[19,20]

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.[21]

Ethylenediaminetetraacetic acid reacts with calcium ions in dentin and demineralizes dentin up to 20 to 30 µm depth in 5 minutes.[22]

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.[23] Marins et al also showed that it produces no genotoxic effect.[24]

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.[25]

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.[25]

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.[26] 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.[27] Using the comet assay, Muller et al found no chromosomal damage.[28] In another study, Hedayati et al showed that incubation of lymphocytes with iodine increased micronuclei frequency and induced genotoxicity.[29]

GENOTOXICITY OF INTRACANAL MEDICAMENTS

Calcium Hydroxide

Calcium hydroxide [$Ca(OH)_2$] was originally introduced to the endodontics in 1920 as a pulp capping material. It is an odorless powder with molecular weight of 74.08.[30]

Calcium hydroxide has low solublility in water, which decreases as the temperature increases. The low solubility of the material is a suitable clinical characteristic.[31] It dissociates into hydroxyl and calcium ions on contact with fluids. In water, $Ca(OH)_2$ may show a thixotropic behavior.[32]

According to Ribeiro et al, $Ca(OH)_2$ cannot promote DNA damage.[33] In another study, Ribeiro et al revealed that it was not able to modulate genotoxicity or even oxidative DNA damage.[34]

Mineral Trioxide Aggregate (MTA)

This material is a combination of refined Portland cement, bismuth oxide and some amounts of K_2SO_4, MgO, SiO_2, CaO, and Na_2SO_4.[35] Portland cement is a combination of tricalcium silicate, dicalcium silicate, gypsum, tricalcium aluminate and tetracalcium aluminoferrite.[36] It has a better working time and has undergone additional processing than regular portland cement.[35] The powder should be mixed with sterile water in a 3:1 powder/liquid ratio.[37] After hydration, MTA solidifies to a hard material in near 3 hours, by absorbing moisture from the surrounding tissues.[37] Hydrated MTA shows initial pH of 10.2, which rises to 12.5 after 3 hours.[38]

Using single cell gel (comet) assay, Ribeiro et al detected no DNA damage after a treatment of cells by MTA for up to 1000 µ/ml concentrations.[39] In another study, Ribeiro et al demonstrated that regular and white MTA did not produce genotoxic effects at 1 to 1000 µg ml^{-1} for 3 hours at 37°C.[40] Another study using CHO cells indicated that MTA is not genotoxic.[41] Braz et al assessed the genotoxic effects of MTA in lymphocytes and failed to detect DNA damage.[42] Camargo et al revealed that MTA cannot negatively influence cell survival.[43] Ding et al showed that MTA and calcium silicate possessed no genotoxic effect.[44] According to Zeferino et al, MTA as well as Portland cement and 15% bismuth oxide were not genotoxic.[45]

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.[46]

Ørstavik and Hongslo concluded that a synthetic polymer based on epoxy-bis-phenol A, induced mutations.[47] 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.[48] Physiological saline eluates of mixed AH26 were not mutagenic.

Leyhausen et al showed that AH-plus revealed no genotoxicity and mutagenicity.[49]

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.[50] 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.[51] Miletic et al found no mutagenicity of AH26 and AH Plus sealers on human lymphocytes in highly controlled conditions *in vitro*.[52]

Formaldehyde is released from some epoxy-based sealers (maximum rate after 48 hours), even though this amount is less than that of paraformaldehyde.[53] The leakage of formaldehyde from the epoxy sealers has been contributed to the mutagenic effects.[48,54]

Formaldehyde is carcinogenic in animals; however, there are only few evidences for showing carcinogenicity in human.[55] 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

1. Cunha SA, Rached FJ Jr, Alfredo E, León JE, Perez DE. Biocompatibility of sealers used in apical surgery: a histological study in rat subcutaneous tissue. Braz Dent J 2011;22(4): 299-305.
2. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-Tetrazolium method. J Endod 2003;29(10):654-657.
3. Visalli G, Baluce B, Maestra SL, Micale RT, Cigano L, De Flora S, Di Pietro A. Genotoxicity damage in the oral mucosa cells of subjects carrying restoratives dental fillings. Arch Toxicol 2012 2013 Jan;87(1):179-187.
4. Kaya A, Undeger U, Aydin S, Omurlu H, Basaran N. Genotoxicity evaluation of dentine bonding agents by comet assay. Int Endod J 2011;44(9):807-816.

5. Protas BW; Kasprzak AM, Kazimierz S. Metal Ions in Life Sciences. Royal Society of Chemistry publishing; 2011. Genotoxicity of metal ions: chemical insights. p. 319-373.
6. Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J 2008;58(6):329-341.
7. Mohammadi Z, Shalavi S. Antimicrobial Activity of Sodium Hypochlorite in Endodontics. J Mass Dent Soc 2013;62(1): 28-31.
8. Hamaguchi F, Tsutsui T. Assessment of genotoxicity of dental antiseptics: ability of phenol, guaiacol, p-phenolsulfonic acid, sodium hypochlorite, p-chlorophenol, m-cresol or formaldehyde to induce unscheduled DNA synthesis in cultured Syrian hamster embryo cells. Jpn J Pharmacol 2000; 83(3):273-276.
9. Hagiwara M, Watanabe E, Barrett JC, Tsutsui T. Assessment of genotoxicity of 14 chemical agents used in dental practice: ability to induce chromosome aberrations in Syrian hamster embryo cells. Mutat Res 2006;603(2):111-120.
10. Aubut V, Pommel L, Verhille B, Orsière T, Garcia S, About I, Camps J. Biological properties of a neutralized 2.5% sodium hypochlorite solution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109(2):e120-125.
11. Marins JS, Sassone LM, Fidel SR, Ribeiro DA. In vitro genotoxicity and cytotoxicity in murine fibroblasts exposed to EDTA, NaOCl, MTAD and citric acid. Braz Dent J 2012;23(5):527-533.
12. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J 2009;42(4):288-302.
13. Gomes BP, Vianna ME, Zaia AA, Almeida JF, Souza-Filho FJ, Ferraz CC. Chlorhexidine in endodontics. Braz Dent J 2013;24(2):89-102.
14. Ribeiro DA, Scolastici C, De Lima PL, Marques ME, Salvadori DM. Genotoxicity of antimicrobial endodontic compounds by single cell gel (comet) assay in Chinese hamster ovary (CHO) cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99(5):637-640.
15. Yeung SY, Huang CS, Chan CP, Lin CP, Lin HN, Lee PH, Jia HW, Huang SK, Jeng JH, Chang MC. Antioxidant and prooxidant properties of chlorhexidine and its interaction with calcium hydroxide solutions. Int Endod J 2007;40(11):837-844.
16. Li YC, Kuan YH, Lee SS, Huang FM, Chang YC. Cytotoxicity and genotoxicity of chlorhexidine on macrophages in vitro. Environ Toxicol 2012;29(4):452-458.
17. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, et al. A new solution for the removal of the smear layer. J Endod 2003;29(3):170-175.
18. Marins JS, Sassone LM, Ribeiro DA. BioPure MTAD induces DNA damage but not cellular death: an in vitro study. Eur J Dent 2009;3(4):285-289.
19. Wiberg E, Wiberg N. Inorganic Chemistry. San Diego: Academic Press; 2001. 1884 p.
20. Harris, Daniel C. Quantitative Chemical Analysis. 7th ed. New York: WH Freeman and Company; 2007. 719 p.
21. Mohammadi Z, Shalavi S, Jafarzadeh H. Ethylenediaminetetraacetic acid (EDTA) in endodontics: a review. Eur J Dent 2013; 7(1 suppl):S135-142.
22. Mohammadi Z, Shalavi S, Gutmann JL. An update on the root canal irrigation solutions. Chonnam Med J (in Press).
23. Heindorff K, Aurich O, Michaelis A, Rieger R. Genetic toxicology of ethylenediaminetetraacetic acid (EDTA). Mutat Res 1983;115(2):149-173.
24. Marins JS, Sassone LM, Fidel SR, Ribeiro DA. In vitro genotoxicity and cytotoxicity in murine fibroblasts exposed to EDTA, NaOCl, MTAD and citric acid. Braz Dent J 2012;23(5):527-533.
25. Mohammadi Z. Iodine compounds in endodontics: an update review. Dent Today 2009;28(6):58, 60-63.
26. Poul JM, Huet S, Godard T, Sanders P. Lack of genotoxicity of potassium iodate in the alkaline comet assay and in the cytokinesis-block micronucleus test. Comparison to potassium bromate. Food Chem Toxicol 2004;42(2):203-209.
27. Hikiba H, Watanabe E, Barrett JC, Tsutsui T. Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. J Pharmacol Sci 2005;97(1):146-152.
28. Müller G, Hai DN, Kramer A. Lack of in vitro genotoxicity of povidone-iodine in solution, in ointment or in a liposomal formulation (Repithel). Dermatology. 2006;212 (Suppl 1): 94-97.
29. Hedayati M, Shafaghati N, Hosseinimehr SJ. Resveratrol mitigates genotoxicity induced by iodine-131 in primary human lymphocytes Radiat Environ Biophys 2013;52(2): 287-291.
30. Mohammadi Z, Dummer P. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J 2011;44(8):697-730.
31. Farhad A, Mohammadi Z. Calcium hydroxide: a review. Int Dent J 2005;55(5):293-301.
32. Mohammadi Z, Shalavi S, Yazdizadeh M. Antimicrobial activity of calcium hydroxide in endodontics: a review. Chonnam Med J 2012;48(3):133-140.
33. Ribeiro DA, Marques ME, Salvadori DM. Lack of genotoxicity of formocresol, paramonochlorophenol, and calcium hydroxide on mammalian cells by comet assay. J Endod 2004;30(8):593-596.
34. Ribeiro DA, Marques ME, Salvadori DM. Antimicrobial endodontic compounds do not modulate alkylation-induced genotoxicity and oxidative stress in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102(2):e32-36.
35. Parirokh M, Torabinejad M. Mineral trioxide aggregate: A comprehensive literature review-Part 1: chemical, physical and antibacterial properties. J Endod 2010;36(1):16-27.
36. Darvell BW, Wu RC. 'MTA'—an Hydraulic Silicate Cement: review update and setting reaction. Dent Mater 2011;27: 407-422.
37. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999;25(3):197-205.
38. Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endod 1995;21(7):349-353.
39. Ribeiro DA, Duarte MA, Matsumoto MA, Marques ME, Salvadori DM. Biocompatibility in vitro tests of mineral trioxide aggregate and regular and white Portland cements. J Endod 2005;31(8):605-607.
40. Ribeiro DA, Matsumoto MA, Duarte MA, Marques ME, Salvadori DM. In vitro biocompatibility tests of two commercial types of mineral trioxide aggregate. Braz Oral Res 2005;19(3):183-187.
41. Ribeiro DA, Sugui MM, Matsumoto MA, Duarte MA, Marques ME, Salvadori DM. Genotoxicity and cytotoxicity of mineral trioxide aggregate and regular and white. Portland cements on Chinese hamster ovary (CHO) cells in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101(2): 258-261.

42. Braz MG, Camargo EA, Salvadori DM, Marques ME, Ribeiro DA. Evaluation of genetic damage in human peripheral lymphocytes exposed to mineral trioxide aggregate and Portland cements. J Oral Rehabil 2006;33(3):234-239.
43. Camargo SE, Camargo CH, Hiller KA, Rode SM, Schweikl H, Schmalz G. Cytotoxicity and genotoxicity of pulp capping materials in two cell lines. Int Endod J 2009;42(3):227-237.
44. Ding SJ, Kao CT, Chen CL, Shie MY, Huang TH. Evaluation of human osteosarcoma cell line genotoxicity effects of mineral trioxide aggregate and calcium silicate cements. J Endod 2010; 36(7):1158-1162.
45. Zeferino EG, Bueno CE, Oyama LM, Ribeiro DA. Ex vivo assessment of genotoxicity and cytotoxicity in murine fibroblasts exposed to white MTA or white Portland cement with 15% bismuth oxide. Int Endod J 2010;43(10):843-848.
46. Dahl JE. Toxicity of endodontic filling materials. Endod Topics 2005:12(1):39-43.
47. Ørstavik D, Hongslo JK. Mutagenicity of endodontic sealers. Biomater 1985;6(2):129-132.
48. Schweikl H, Schmalz G, Stimmelmayr H, Bey B. Mutagenicity of AH26 in an in vitro mammalian cell mutation assay. J Endod 1995;21(8):407-410.
49. Leyhausen G, Heil J, Reifferscheid G, Waldmann P, Geurtsen W. Genotoxicity and cytotoxicity of the epoxy resin-based root canal sealer AH plus. J Endod 1999;25(2):109-113.
50. Ersev H, Schmalz G, Bayirli G, Schweikl H. Cytotoxic and mutagenic potencies of various root canal filling materials in eukaryotic and prokaryotic cells in vitro. J Endod 1999; 25(5):359-363.
51. Tai KW, Huang FM, Huang MS, Chang YC. Assessment of the genotoxicity of resin and zinc-oxide eugenol-based root canal sealers using an in vitro mammalian test system. J Biomed Mater Res 2002;59(1):73-77.
52. Miletić I, Jukić S, Anić I, Zeljezić D, Garaj-Vrhovac V, Osmak M. Examination of cytotoxicity and mutagenicity of AH26 and AH Plus sealers. Int Endod J 2003;36(5):330-335.
53. Spångberg LS, Barbosa SV, Lavigne GD. AH26 releases formaldehyde. J Endod 1993:19(12):596-598.
54. Ersev H, Schmalz G, Bayirli G, Schweikl H. Cytotoxic and mutagenic potencies of various root-canal filling materials in eukaryotic and prokaryotic cells in vitro. J Endod 1999:25 (5):359-363.
55. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans and World Health Organization and International Agency for Research on Cancer [Internet]. Reevaluation of some Organic Chemicals, Hydrazine and Hydrogen Peroxide. New York: WHO Publications Centre; 1999. Available at: http://apps.who.int/bookorders/anglais/detart1.jsp?sessl an=1&codlan=1&codcol=72&codcch=71