

Cytotoxicity of two epoxy resin based root canal sealers using the ^{51}Cr -release method

Abstract

The cytotoxicity of fresh samples of AH26 and AH Plus were tested using the ^{51}Cr -release method at 12 and 72 h. Heteroploid L929 mouse fibroblasts were used as target cells. For the ^{51}Cr -release assay, cells were labeled with ^{51}Cr before exposure to the test materials, and the radioactivity in the supernatant was counted with a gamma particle counter. The difference in cytotoxicity between the two materials was evaluated using the Wilcoxon Sum-Rank Test. The difference between AH26 and AH Plus was not statistically significant at 12h ($p=0.8345$) and at 72h ($p=0.676$). Both AH26 and AH Plus had gamma particle readings that were significantly lower than the positive control (formocresol) and significantly higher than the negative control (labeled, but material free cell suspension), as assessed by Wilcoxon Sum-Rank Tests.

Keywords: cytotoxicity, ^{51}Cr -release, resin, AH26, AH plus

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Introduction

The biocompatibility of a root canal sealer plays a significant role in the success of endodontic treatment.¹ The components of these materials can act as general toxins or as specific toxins which target certain metabolic pathways. In both instances, cellular injury can lead to tissue damage or delay and impede tissue repair.

Several *in vitro* methods have been recommended for the evaluation of cytotoxicity of endodontic materials.² The American National Standards Institute, the American Dental Association, the Technical Report ISO-TR 7405 of the International Standards Organization Committee concerned with dentistry (TC 106) and FDI have published guidelines for material evaluation that encourage the use of *in vitro* methods.³⁻⁵

The radiochromium (^{51}Cr) release method was introduced by Spangberg.⁶ According to the method, cells are labeled with ^{51}Cr in the form of sodium chromate, which binds with the intracellular proteins. Toxicity results in cellular changes and the release of the radioactive chromium, which is proportional to the cytotoxic effect of the particular material.

A variety of root canal sealers are currently available and include zinc oxide-eugenol, calcium hydroxide and epoxy resin-based formulations. More recently, a new epoxy resin-based material, AH Plus, has been introduced. AH Plus has been claimed to be an improved paste to paste material based on epoxy amine resins.⁷ Because of its non-formaldehyde releasing formulation, AH Plus has a less cytotoxic effect than AH26. Compared with studies carried out on AH26, there have been a limited number of studies investigating the cytotoxicity of AH Plus.⁸ The purpose of the present study was to compare the cytotoxicity of these two epoxy resin-based root canal sealers using the ^{51}Cr release method.

Materials and methods

The study used 16×10^6 heteroploid L929 mouse fibroblasts in 40ml Eagles' MEM (minimum essential medium) which contained 10% fetal bovine serum (FBS; HyClone, Logan, Utah), 10,000 units of penicillin-G/ml, 10mg of Streptomycin/ml, and 200mM of L-glutamine (Sigma Chemical Co., St Louis). Cells were maintained

at 37°C under 5% CO_2 , 100% humidity, and were labeled with $300\mu\text{Ci}$ ^{51}Cr in the form of isotonic sodium chromate solution for 24h. The labeled cells were rinsed three times with phosphate buffer solution (PBS) to eliminate unbound ^{51}Cr remnants. The rinsed cells were diluted with PBS as 4×10^5 cells in one milliliter. Subsequently, 0.5mg of fresh mixture of each endodontic test material, AH26 (DeTrey, Dentsply, Germany) and AH Plus (DeTrey, Dentsply) were placed into five different test tubes for 12h and five test tubes for 72h. In total, twenty test tubes were prepared with samples weighted under aseptic conditions according to the manufacturers' directions.

One positive and one negative control were prepared for each test tube. Labeled, but material free cell suspension was used as negative control and labeled cell suspension that contains 0.5ml formocresol (Sultan Co. UK) was used as positive control group.

All the test sample tubes were placed on a homogenisator, and later 1ml of cell culture suspension from each test sample tube was centrifuged at 500xg for 8 minutes, thereafter 0.5ml of supernatants were counted with gamma particle counter (Wallac 1480 Wizard 3 Gamma Counter). Overall differences among the groups in ^{51}Cr release at 12h and 72h were evaluated using the Kruskal-Wallis test. The Wilcoxon Sum-Rank test was applied to assess significant pairwise comparisons in ^{51}Cr release between each test material and the control groups. Differences within test groups, between 12 and 72 hour readings, were evaluated using the Wilcoxon Signed-Rank test.

Results

The results at 12 and 72 hours are presented in Table 1 and 2, respectively, and in Figure 1. Overall differences were noted in gamma particle readings for the sealer and control groups at 12 h and 72 h (Kruskal-Wallis Test, $p=0.001$). AH26 and AH Plus had comparable results which were significantly lower than those of the positive control and significantly higher than those of the negative control samples (Wilcoxon Sum-Rank test, $p<0.05$). There was not any significant difference between AH26 and AH Plus at 12 ($p=0.834$) and 72 h ($p=0.676$). There was a borderline significant increase in ^{51}Cr release from 12 to 72 hours for both AH26 and AH plus (Wilcoxon Signed-Rank test, $p=0.062$).

Table 1 Gamma counter readings at 12 h.

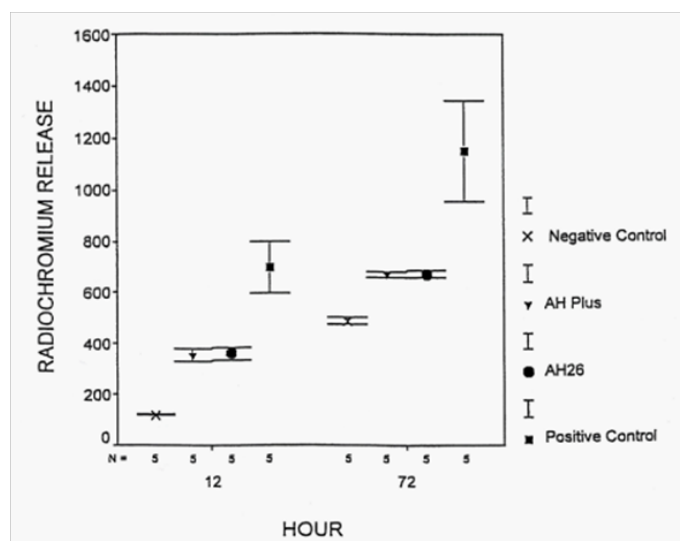
	TEST SAMPLES					Mean	SD
	1	2	3	4	5		
ROOT CANAL SEALERS							
AH26	336.5	378.6	362.2	341.7	381.2	360.0	20.54
AH Plus	360.1	339.7	384.1	337.9	344.6	353.3	19.32
(-) Control	122.1	117.7	120.8	115.0	123.3	119.8	3.38
(+) Control	653.7	789.7	620.2	641.3	780.4	697.1	81.28

SD: Standard Deviation; (-) Control: Labeled but Material Free Cells; (+) Control: Formocresol

Table 2 Gamma counter readings at 72 h.

	TEST SAMPLES					Mean	SD
	1	2	3	4	5		
ROOT CANAL SEALERS							
AH26	684.0	660.0	671.0	679.3	663.3	671.5	10.21
AH Plus	666.2	677.1	682.0	661.0	659.0	669.1	10.08
(-) Control	487.5	501.1	492.0	472.2	483.0	487.2	10.71
(+) Control	1330.4	997.8	1271.1	981.0	1172.0	1150.5	157.65

SD, standard deviation; (-) Control, labeled but material free cells; (+) Control, Formocresol

**Figure 1** Cytotoxicity (mean ^{51}Cr scores and 95% confidence intervals) of AH plus and AH26 at 12 and 72 hours.

Discussion

There is not enough information available on the cytotoxic effect of AH plus. The ^{51}Cr assay is extremely sensitive and has a good dose-effect relationship for most toxic agents,⁹ while the level of radiation used during this type of experiment is low.¹⁰ The results of *in vitro* and *in vivo* studies often do not correlate but the cytotoxic induction of any test material in the cell culture medium can be regarded as similar to the living tissues.

Briseno and Willerhausen¹¹ concluded that AH26 showed severe cytotoxic reaction after 24 hours in comparison to Diaket and Endo-

Fill. The same conclusion has been supported by other studies.^{12–15} It is possible that AH26 releases formaldehyde during the setting reaction which is responsible for the toxicity of AH26. Similar to our results, Jukic et al.¹⁶ evaluated that both, AH26 and AH plus were cytotoxic and that AH plus was more toxic than AH26.

Based on the chromium release method and procedure used in the present study, AH26 and AH Plus have the same degree of cytotoxic effect at 12 and 72h. The adequate combination of sealing ability and biocompatibility of a root canal sealer is important for a favorable prognosis in root canal treatment. Since *in vitro* toxicity studies are scarce and the dynamic nature of human periapical tissues cannot easily be simulated *in vitro*, further studies are needed to evaluate the toxicity of sealing materials in endodontics.

Acknowledgments

None.

Conflict of interest

The author declares that there is no conflict of interest.

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