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Research Article

An Assessment of the Neurotoxicity of Different Root Canal Sealers on Rat Sciatic Nerve: An Electrophysiologic and Histopathologic Study

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Abstract

Objective: The objective of this study was to compare the neurotoxic effect of Endosequence BC sealer and MTA FilLapex sealer versus AH-plus by assessing the nerve conduction velocity (NCV) of isolated rat sciatic nerve and features of histopathological neurotoxic effect.

Methods: Fifty seven white female albino rats were used in this study and grouped into three equal groups, 19 rats for each sealer group. All sealers were prepared according to manufacturer's instruction and applied on the all entire length of the isolated rat sciatic nerve. The neurotoxicity of the root canal sealers (RCSs) was assessed by calculating the (NCV) of isolated rat sciatic nerve before, after 5 minutes and after 30 mins of sealer application on the nerve by the electrophysiologic data acquisition system (power lab) device. NCV can determine the nerve destruction and damage. Sciatic nerve samples that revealed a significant decrease in NCV due to severe neurotoxic effects had been selected for subsequent histopathological analysis.

Results: The only statistical significant difference of the NCVs between groups was observed after 30 minutes between AH-Plus and Endosequence and between AH-Plus and MTA Fillapex so AH-Plus has the most neurotoxic effects followed by MTA Fillapex and the least one was Endosequence bc Histological examination of haematoxylin and eosin (H&E) stained sections of sciatic nerves with a significant decrease in NCV revealed that there were marked histopathological changes for AH-plus group compared to Endosequence BC and MTA Fillapex groups. Due to the very limited research papers on the neurotoxicity of the endodontic materials we conducted our study. Conclusion: Overextension with pushing of resin based cement into periapical tissue should be avoided otherwise a paraesthesia might occurs moreover, measuring the NCV was proved to be a valuable tool in assessing the neurotoxic effect of the root canal sealer and Histopathological data are essential tool to confirm the electrophysiological findings.

Conclusion: Overextension with pushing of resin based cement into periapical tissue should be avoided otherwise a paraesthesia might occurs moreover, measuring the NCV was proved to be a valuable tool in assessing the neurotoxic effect of the root canal sealer and Histopathological data are essential tool to confirm the electrophysiological findings.

Clinical Significance: Apical extrusion of endodontic filling materials may cause Inflammation, nerve toxicity and damage so; extrusion of root canal filling should be avoided during endodontic treatment

Keywords: Neurotoxicity; Root Canal Sealer; AH-Plus; Endosequence BC; MTA Fillapex; Rat Sciatic Nerve

Abbreviations

NCV: Nerve Conduction Velocity; RCSs: Root Canal Sealers; MTA: Mineral Trioxide Aggregate; BC: Bioceramic

Introduction

The first priority of effective root canal therapy is to enter, shape and clean the system in a manner that will allow efficient and total filling of the root canal space [1].

Overfill of obturation material into the periradicular tissue has been correlated with foreign body reactions, flare-ups, and increased postoperative discomfort [2].

Endodontic sealers are an essential part of obturation; not only they provide for an adequate seal between the core material and the dentinal walls, but they are also effective antimicrobials inside the canal system [3].

Extrusion of root canal sealers during obturation may cause damage to the surrounding anatomic structures with Clinical symptoms like pain, swelling and paresthesia or anesthesia may be present [4].

It is the sealers and its components that are recognized by the scientific literature as neurotoxic or highly irritating when extruded from the root canal which affect the related nerve tissue [5].

There is a direct relationship between the amount of time that the material remains on the nerve and immediate management to reduce the risk of permanent damage [6].

Materials and Methods

Materials

Three RCSs (AH-Plus, Endosequence bc and MTA fillapex) were tested. Composition and manufacturer of tested sealers are listed in table 1.

Material	Composition	manufacturer
AH-Plus	Paste A: Bisphenol-A and -F epoxy resins, calcium tungstate, zirconium oxide, silica, iron oxide pigments. Paste B: Amines, calcium tungstate, zirconium oxide, silica, silicone oil.	(Dentsply DeTrey GmbH, Konstanz, Germany
Endosequence BC	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agent	(Brasseler, Savannah, Georgia, USA)
MTA fillapex	Paste A: salicylate resin, bismuth trioxide and fumed silica. Paste B: mineral trioxide aggregate 40%, fumed silica. Titanium dioxide and base resin.	(Angelus Ind ustria de Produtos Odontol ogicos S/A, Londrina, PR, Brazil)

Table 1: Composition and manufacturing of tested sealers.

Surgical procedures

Fifty seven white female Albino rats were killed by cervical dislocation as a method of human euthanasia (Figure 1A). All the experimental procedures were carried out according to the protocol reviewed and approved to be in accordance with the guidelines of the ethical committee of Research in Faculty of Oral and Dental Medicine, Cairo University. Each animal was placed flat on its ventral surface on a towel. The whole procedures were done under proper aseptic conditions, using sterile instrument. The lateral aspect of the right thigh, hip and flank was then routinely prepared, including trimming of the hair and disinfection with betadine. The sciatic nerve was uncovered through a posterolateral longitudinal straight incision (Figure 1B) using Bard parker blade no. 15. The entire length of the nerve was made visible. It was uncovered approximately 2 cm of the sciatic nerve. Then the sciatic nerve was dissected from the rat by scissor and grasped by tweezer. The nerve was placed in dish which is filled with tyrode solution (Figure 1C).

All the procedures were conducted in the lab room in Medical Physiology Department, Faculty of Medicine, EL Kaser EL Eini and in the Oral Pathology Department Faculty of Oral and Dental Medicine, Cairo University.

Electrophysiologic analysis

Using forceps, the nerve was lifted out of its dish before sealer application by grasping the end of the nerve and placed on the wire electrodes of nerve chamber (Figure 1D) that contains tyrode solution making sure it is in contact with each of the active connections then the tested sealer was prepared according to manufacturer's instruction and applied on the all entire length of the isolated sciatic nerve by spatula and the cover placed back on the nerve bath.

A bipolar hook electrode was used to stimulate the sciatic nerve and another one for recording the nerve potential.

The recording of nerve potentials was carried out using an electrophysiologic data acquisition system (Power Lab) (Figure 1E).

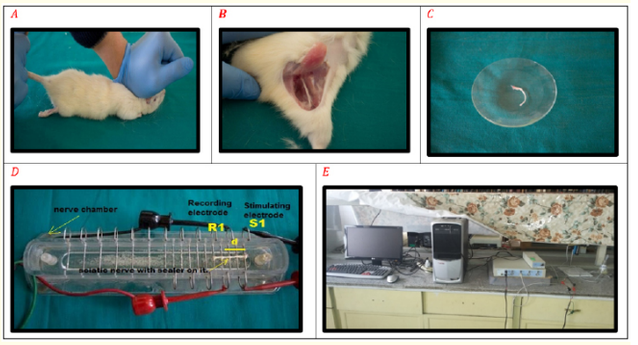

Figure 1: (A) Cervical dislocation of the rat, (B) Exposure of the entire length of the rat sciatic nerve, (C) Isolated rat sciatic nerve in dish filled with tyrode solution, (D) The nerve chamber containing the isolated sciatic nerve, (E) An electrophysiologic data acquisition system (Power Lab).

NCV was calculated by the absolute method, which means that the velocity was calculated using a single latency and distance measurement by dividing distance between the stimulating and recording electrodes(d) by the average latency difference between the onsets of compound action potentials Velocity = d/latency (m/s) or (mm/ms) [7]

Histopathological analysis

Sciatic nerve samples that revealed a significant decrease in NCV due to severe neurotoxic effects had been selected for subsequent histopathological analysis; they have been placed in labelled jars. Sciatic nerve tissue samples immersed to 10% buffered formalin for 24 hours at room temperatures, and then fixed tissues were washed in running tap water for 4 - 8 hours. Nerve tissues were embedded in paraffin and blocked, the sections were cut at thickness 4 - 5 micrometer with microtome and were prepared on glass slides for analysis.

Sections were stained with haematoxylin and eosin stain and were examined under light microscope. Evaluation of all groups was done with regard to: Axon and epineurium degeneration, thickening degree of epineurium and perineurium, fibrosis, lymphocyte Infiltration, edema and vacuolization.

Statistical analysis

Data were coded and entered using the statistical package SPSS (statistical package for the social science) version 22. Data was summarized using mean and standard deviation in quantitative data. Comparisons between groups were done using ANOVA with Post Hoc test (Chan, 2003a). For comparison of serial mea-

surements within each group repeated measures ANOVA was used (Chan, 2004). P-values less than 0.05 were considered as statistically significant.

Results and Discussion

Electrophysiologic findings

The only statistical significant difference of the NCVs between groups was observed after 30 mins between AH-Plus and Endosequence and between AH-Plus and MTA Fillapex (Figure 2,3) so AH-Plus has the most neurotoxic effects followed by MTA Fillapex and the least one was Endosequence BC (Table 2).

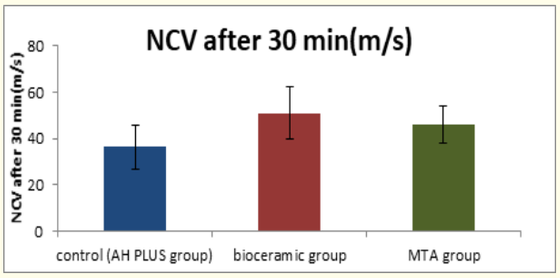

Figure 2: Bar chart showing the mean value of NCV of all groups after 30 minutes of the sealer application on the nerve tissues.

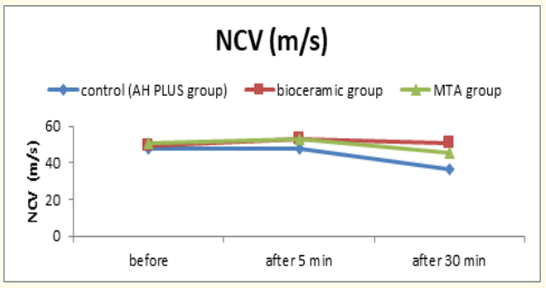

Figure 3: Diagram showing the time course of mean value of the NCV of all groups.

Groups NCV	Control (AH PLUS group)	Endosequence group	MTA group	Inter-group P value
Before sealer application	47.84 ± 13.76	49.58 ± 8.70	50.47 ± 9.50	0.751
After 5 minutes	47.42 ± 7.70	53.05 ± 10.09	52.79 ± 9.14	0.105
After 30 minutes	36.32 ± 9.51	50.95 ± 11.15 *	45.89 ± 8.08 *	< 0.001

Table 2: Descriptive statistics and test of significance showing Comparison between groups at different periods.

*: statistically significant compared to corresponding value in control group (P < 0.05).

Histological findings

Histological examination of haematoxylin and eosin (H&E) stained sections of sciatic nerves with a significant decrease in NCV revealed that there were marked histopathological changes for AH-plus group compared to Endosequence bc and MTA Fillapex groups (Figure 4).

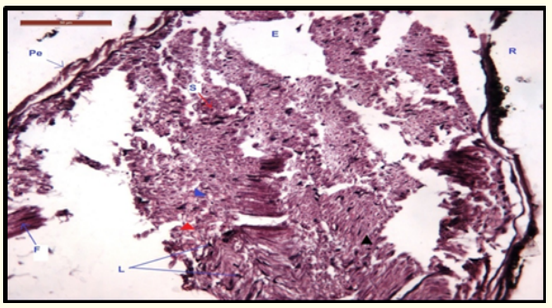

Figure 4: Photomicrograph of nerve tissues section of AH-plus control group, Pe: Perineurium Degeneration; R: Residual Sealer Substance; S: Schwann Cell; E: Edema; F: Fibrosis; L: Lymphocyte Infiltration; Red Arrow Head: Axonal Degeneration; Blue Arrow Head: Vacuolization; Black Arrow Head: Myelin Sheath Degeneration; (H&Ex200).

There is a close relation between the root canal apex and the inferior alveolar nerve(IAN) bundle, the distance between the anatomical root apex and the inferior alveolar nerve was equal or less than 3 mm [8].

The spongy bone in the molar area has many vacuoles which facilitates the spread of irrigation products and filling material toward the inferior alveolar neurovascular set [9,10].

The sealers have direct effect on trigeminal nociceptors activation leading to strong release of calcitonin gene-related peptide, and may therefore lead to pain and neurogenic inflammation [2].

Rats are mammals that share many processes with humans and are appropriate for use to answer many research questions, available and generally mild-tempered and docile, making them easy for researchers to handle [11].

In our study, the neurotoxic effects of AH-Plus, Endosequence and MTA fillapex sealers were evaluated on rat sciatic nerve tissue because it was a mixed nerve (a nerve composed of both sensory and motor fibers), similar to the IAN, easy to expose surgically without causing any damage, and had a thick enough nerve bundle [12] to facilitate electrophysiologic recordings [13].

The rats were killed by cervical dislocation as a method of human euthanasia because there is no need for the rats after sciatic nerve isolation [14].

The maximum application period of the sealer on the isolated sciatic nerve was 30 minutes as increasing the time of application will endanger the nerve viability and hence give false measurements and this was consistent with the present pilot study.

When the root canal sealers were contacted with a nerve tissue, it has been reported that the sealers affect nerve transmission in 2 ways. The first way is that ionic imbalance in the extracellular fluid surrounding the axons can lead to temporary conduction block; this state is reversible and recovery is fast and complete. The second way, which is more severe, is where chemical damage occurred by time leads to axonal degeneration in the distal region

of the damaged area and impulse conduction ceases. In this type of injury, recovery is slow, and complete recovery rarely occurs for this reason [13].

Due to the very limited research paper on the neurotoxicity of the endodontic materials and from the point that cytotoxicity is the quality of being toxic to cells and the nerve cells are much like other cells [15] a link could be established between cytotoxicity and neurotoxicity that is to say any cytotoxic material is probably a neurotoxic one as well.

AH-plus sealer can cause toxic effects when extruded from the root canal apex. It is well documented that AH-plus sealer has moderate to severe cytotoxic effect on the fibroblast cells [16]. AH-plus sealer exhibited a variable degree of neurotoxic effect on rat sciatic nerves [13] and on the primary neurons of orofacial tissues [17].

AH-plus sealer can cause toxic effects when extruded into the mandibular canal due to the release of small amount of formaldehyde and its cytotoxic bisphenol component [18].

AH-plus sealer release minimal levels of formaldehyde during their setting reaction, but do not contain formaldehyde as an ingredient [19].

The formaldehyde produced from root canal fillings may cause permanent damage of the nerve [20] and are reported to be the agents that most commonly cause neurotoxic reactions [12].

Bisphenol A diglycidyl ether was identified as a mutagenic and cytotoxic component of resin-based materials [21].

Several previous studies showed that MTA Fillapex strongly affected cell viability and has cytotoxic properties [22-24].

The observed cytotoxicity of MTA Fillapex and hence its neurotoxicity can be explained by its chemical composition which is composed of two pastes, one containing MTA and the other containing salicylate resin [24].

Salicylate resin component of MTA Fillapex has stimulated the process of apoptosis in human fibrosarcoma and has caused the fragmentation of cell genetic material [25].

Arsenic and lead components could be related to MTA Fillapex toxicity [26,27].

Bioceramic sealers will not result in a significant inflammatory response if an overfill occurs during the obturation process [28] Endosequence BC sealer exhibited a cytotoxic effect on fibroblasts *in vitro* studies [24,29,30].

The neurotoxic effects of Endosequence bc sealer were showed by Kilkis., *et al.* 2015 [14] and Er., *et al.* 2017 [18]. The long setting time of Endosequence bc may be responsible for some components of the material to leach for extended periods of time and influence adversely cell viability, which may also explain its cytotoxicity and neurotoxicity [31]. Alkaline pH may also explain the toxic properties of Endosequence BC sealer [17].

The results of this study were in accordance with Kilkis., et al. 2015 [14] who reported that NCV was statistically reduced in AH-plus group compared to bioceramic sealer group.

In agreement with our study Er., *et al.* 2017 [18] showed that AH-plus sealer has more neurotoxic effects on cultured rat trigeminal ganglion neurons than iRoot SP (Endosequence BC sealer), Barbra., *et al.* [25] proved that MTA fillapex showed more cytotoxic effect in comparison to the Endosequence BC sealer and Zoufan., *et al.* 2011 showed that Endosequence BC sealer have lower cytotoxicity than AH-plus sealer.

The results of the present study were inconsistent with that of Zhou., *et al.* [30] who showed that MTA fillapex was more cytotoxic than both Endosequence BC sealer and AH-plus.

Contrary to the present study Leyhausen., *et al.* 1999 and Camps and About 2003 reported that AH-Plus sealers caused mild to no cellular damage.

Silva., *et al.* 2016 showed that MTA fillapex and AH-plus had similar toxicity in fresh conditions but MTA fillapex more cytotoxic than AH-plus in the set form and this mainly due to the high solubility of MTA fillapex.

Histopathological evaluation of the dissected nerves was done because nerve biopsy is a valuable tool in the diagnosis of neuropathies [32] and the histopathological findings matched to the electrophysiological results with regard to marked (axon and epineurium degeneration, thickening degree of epineurium and perineurium, fibrosis, lymphocyte infiltration, edema and vacuolization) of AH-Plus group compared to MTA fillapex and Endosequence groups.

Conclusion

Overextension with pushing of resin based cement into periapical tissue should be avoided otherwise a paraesthesia might occurs moreover, measuring the NCV was proved to be a valuable tool in assessing the neurotoxic effect of the root canal sealer and histopathological data are essential tool to confirm the electrophysiological findings.

Conflict of Interest

The authors have no personal or financial conflicts of interests regarding this manuscript.

Bibliography

1. Gluskin AH. "Anatomy of an overfill: a reflection on the process". *Endodontic Topics* 16.1 (2007): 64-81.

2. Ruparel NB., *et al*. "Direct effect of endodontic sealers on trigeminal neuronal activity". *Journal of Endodontics* 40.5 (2014): 683-687.

3. D'SOUZA Leslie Henston Joe., *et al*. "Root Canal Sealers & its Role in Successful Endodontics-A Review". *Annals of Dental Research* 2.2 (2014): 70-79.

4. Daloupoulou Athina., *et al*. "Extrusion of Root Canal Sealer in Periapical Tissues-Report of Two Cases with Different Treatment Management and Literature Review". *Balkan Journal of Dental Medicine* 21.1 (2017): 12-18.

5. Gambarini G., *et al*. "Differential diagnosis of endodontic-related inferior alveolar nerve paraesthesia with cone beam computed tomography: a case report". *International Endodontic Journal* 44.2 (2011): 176-181.

6. Alves FR., *et al*. "Endodontic-related facial paresthesia: systematic review". *Journal of the Canadian Dental Association* 80 (2014): e13.

7. Alkan A., *et al*. "The effects of hemostatic agents on peripheral nerve function: an experimental study". *Journal of Oral and Maxillofacial Surgery* 65.4 (2007): 630-634.

8. Chong BS., *et al*. "The anatomical relationship between the roots of mandibular second molars and the inferior alveolar nerve". *International Endodontic Journal* 48.6 (2014): 549-555.

9. Tilotta-Yasukawa F., *et al*. "Labiomandibular paresthesia caused by endodontic treatment: an anatomic and clinical study". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 102.4 (2006): e47-e59.

10. Knowles KI., *et al*. "Paresthesia associated with endodontic treatment of mandibular premolars". *Journal of Endodontics* 29.11 (2003): 768-770.

11. Bateson P. "Assessment of pain in animals". *Animal Behaviour* 42 (1991): 827-839.

12. Iannaccone PM and Jacob HJ. "Rats". *Disease Models and Mechanisms* 2.5-6 (2009): 206-210.

13. Serper A., *et al*. "Comparative neurotoxic effects of root canal filling materials on rat sciatic nerve". *Journal of Endodontics* 24.9 (1998): 592-594.

14. Kılkış BT., *et al*. "Neurotoxicity of various root canal sealers on rat sciatic nerve: an electrophysiologic and histopathologic study". *Clinical oral investigations* 19.8 (2015): 2091-2100.

15. A Fritsch. "Standard Euthanasia Guidelines - Use of Cervical Dislocation for Rodents" (2014): 1-3.

16. Siegelbaum SA and Hudspeth AJ. "Principles of neural science". ER Kandel, JH Schwartz, and TM Jessell (Eds.). New York: McGraw-hill volume 4 (2000): 1227-1246.

17. Karapınar-Kazandağ M., *et al*. "Cytotoxicity of 5 endodontic sealers on L929 cell line and human dental pulp cells". *International Endodontic Journal* 44.7 (2011): 626-634.

18. Er K., *et al*. "Neurotoxicity evaluation of three root canal sealers on cultured rat trigeminal ganglion neurons". *Journal of Clinical and Experimental Dentistry* 9.1 (2017): e34-e39.

19. Cohen BI., *et al*. "Formaldehyde evaluation from endodontic materials". *Oral Health* 88.12 (1998):37-39.

20. Leonardo MR., *et al*. "Release of formaldehyde by 4 endodontic sealers". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 88.2 (1999): 221-225.

21. El Sayed F., *et al*. "Contact urticaria from formaldehyde in a root canal dental paste". *Contact Dermatitis* 33.5 (1995): 353.

22. Roggendorf M. Bayerisches Zahnärzteblatt. Sept. München Germany, Bavarian Dental Journal (2004): 324.

23. Bin CV., *et al*. "Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate". *Journal of Endodontics* 38.4 (2012): 495-500.

24. Silva EJNL., *et al*. "Evaluation of cytotoxicity and up-regulation of gelatinases in human fibroblast cells by four root canal sealers". *International Endodontic Journal* 45.1 (2012): 49-56.

25. Baraba A., *et al*. Cytotoxicity of two bioactive root canal sealers". *Acta Stomatologica Croatica* 50.1 (2016): 8-13.

26. Mahdi JG., *et al*. "Calcium salicylate mediated apoptosis in human HT-1080 fibrosarcoma cells". *Cell Proliferation* 39.4 (2006): 249-260.

27. De-Deus G., *et al*. "Negligible expression of arsenic in some commercially available brands of Portland cement and mineral trioxide aggregate". *Journal of Endodontics* 35.6 (2009): 887-890.

28. Borges RP., *et al*. "Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test". *International Endodontic Journal* 45.5 (2012): 419-428.

29. Koch MJ. "Formaldehyde release from root-canal sealers: influence of method". *International Endodontic Journal* 32.1 (1999): 10-16.

30. Zhou H., *et al*. "In vitro cytotoxicity of calcium silicate-containing endodontic sealers". *Journal of Endodontics* 41 (2015): 56-61.

31. Zhang W., *et al*. "Ex vivo cytotoxicity of a new calcium silicate-based canal filling material". *International Endodontic Journal* 43.9 (2010): 769-774.

32. Weis J., *et al*. "Processing of nerve biopsies: a practical guide for neuropathologists". *Clinical Neuropathology* 31.1 (2012): 7-23.

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