

Original Article

Interaction between lidocaine hydrochloride (with and without adrenaline) and various irrigants: A nuclear magnetic resonance analysis

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ABSTRACT

Background: Interaction between local anesthetic solution, lidocaine hydrochloride (with and without adrenaline), and root canal irrigants such as sodium hypochlorite (NaOCl), ethylene diamine tetra-acetic acid (EDTA), and chlorhexidine (CHX) has not been studied earlier. Hence, the purpose of this *in vitro* study was to evaluate the chemical interaction between 2% lidocaine hydrochloride (with and without adrenaline) and commonly used root canal irrigants, NaOCl, EDTA, and CHX.

Materials and Methods: Samples were divided into eight experimental groups: Group I-Lidocaine hydrochloride (with adrenaline)/3% NaOCl, Group II-Lidocaine hydrochloride (with adrenaline)/17% EDTA, Group III- Lidocaine hydrochloride (with adrenaline)/2% CHX, Group IV-Lidocaine hydrochloride (without adrenaline)/3% NaOCl, Group V-Lidocaine hydrochloride (without adrenaline)/17% EDTA, Group VI-Lidocaine hydrochloride (without adrenaline)/2% CHX, and two control groups: Group VII-Lidocaine hydrochloride (with adrenaline)/deionized water and Group VIII-Lidocaine hydrochloride (without adrenaline)/deionized water. The respective solutions of various groups were mixed in equal proportions (1 ml each) and observed for precipitate formation. Chemical composition of the formed precipitate was then analysed by nuclear magnetic resonance spectroscopy (NMR) and confirmed with diazotation test.

Results: In groups I and IV, a white precipitate was observed in all the samples on mixing the respective solutions, which showed a color change to reddish brown after 15 minutes. This precipitate was then analysed by NMR spectroscopy and was observed to be 2,6-xylydine, a reported toxic compound. The experimental groups II, III, V, and VI and control groups VII and VIII showed no precipitate formation in any of the respective samples, until 2 hours.

Conclusion: Interaction between lidocaine hydrochloride (with and without adrenaline) and NaOCl showed precipitate formation containing 2,6-xylydine, a toxic compound.

Key Words: Lidocaine hydrochloride, precipitate, sodium hypochlorite, 2,6-xylydine

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INTRODUCTION

The goal of endodontic therapy is to eliminate microorganisms from the infected root canal system. Mechanical cleaning and shaping of the root canal greatly reduces the microbial load,^[1] but because of the complex anatomy, organic, and inorganic residues including bacteria cannot be completely removed from the canal and do often persist.^[2] Hence, chemical

debridement in the form of various irrigants is required in conjunction with mechanical preparation of the root canal system for achieving optimal results.^[3]

The ideal irrigant used for root canal therapy should possess adequate tissue dissolving property with lubricating action, prolonged antimicrobial effect, be non-toxic, non-allergenic, and be an effective germicide and fungicide.^[4] No single irrigant can perform all the desired actions and hence, usually a combination of irrigants is employed during root canal therapy.^[5]

The most commonly used irrigant during root canal therapy is sodium hypochlorite (NaOCl) in concentrations ranging from 0.5% to 6%. It is an excellent tissue solvent, and antibacterial agent. The formation of hypochlorous acid on contact with organic debris is responsible for its germicidal ability.^[6] Ethylene diamine tetra-acetic acid (EDTA) is a chelating agent and it removes calcium ions from tooth structure.^[7] EDTA solutions are usually used in concentrations ranging from 10% to 17% for smear layer removal.^[4] However, 17% EDTA has minimal tissue dissolution capacity compared to that of sodium hypochlorite.^[8] Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent, active against Gram-positive and Gram-negative bacteria including yeast cells.^[9] It is commonly used in 2% concentration for root canal disinfection. It also exhibits substantivity resulting in prolonged antimicrobial effect.^[3]

Basrani *et al.*, reported that the interaction between NaOCl and CHX results in the formation of para-chloroaniline precipitate,^[10] a known carcinogen.^[11] In a similar manner, Grande *et al.*, reported the interaction between EDTA and NaOCl by NMR analysis, and concluded that EDTA reduces the free available chlorine content of NaOCl, and hence, its tissue-dissolving capability.^[12]

Nuclear magnetic resonance (NMR) spectroscopy is based on the disturbance of the energy levels of the nuclei influenced by a strong magnetic field. Some nuclei have a certain spin that can be described as the rotation of the nuclei around an axis. A particular nuclear magnetic momentum can be associated to each nucleus. When one of these nuclei is inserted in a homogenous external magnetic field, there is a magnetic interaction between its magnetic momentum and the magnetic field itself. NMR utilizes this interaction to allow the sample to absorb a known wavelength of electromagnetic radiation.^[12]

The most commonly used local anesthetic solution in endodontics is 2% lidocaine hydrochloride with or without adrenaline.^[13] A white precipitate was observed when NaOCl was loaded in a syringe which was used to load local anesthetic solution. It is not known whether this precipitate is toxic or not. Such potential interactions might arise, if lidocaine hydrochloride is administered intra-pulpally, following which NaOCl is used as a tissue solvent.

Until date, the chemical interaction between local anesthetic solution and commonly used root canal irrigants has not been studied. The purpose of this *in vitro* study was to evaluate the chemical interaction between 2% lidocaine hydrochloride (with and without adrenaline) and root canal irrigants, 3% NaOCl, 17% EDTA, and 2% CHX.

MATERIALS AND METHODS

The study was conducted in a laboratory set-up with the root canal irrigants and local anesthetic solution to be assessed. The sample frame included 10 vials of root canal irrigants in each group. A total of 8 groups were assessed for the study, which were the following [Table 1]: Local anesthetic solution, 2% lidocaine hydrochloride with adrenaline (1: 200,000) (Astra Zeneca Pharma India limited, Bangalore, India) and 2% lidocaine hydrochloride without adrenaline (Astra Zeneca Pharma India limited), 3% NaOCl (Vensons India, Bengaluru, India), 2% CHX (Calypso, Septodent Health Care India Pvt. Ltd., Maharashtra, India) and 17% aqueous EDTA liquid (Prime dental Pvt. Ltd., Thane) were used. Deionized water (Aqua shine, SPARK, Chennai, India) was used as control.

Table 1: Experimental and Control groups

Group I	Lidocaine hydrochloride (with adrenaline) with 3% sodium hypochlorite
Group II	Lidocaine hydrochloride (with adrenaline) with 17% EDTA liquid
Group III	Lidocaine hydrochloride (with adrenaline) with 2% chlorhexidine
Group IV	Lidocaine hydrochloride (without adrenaline) with 3% sodium hypochlorite
Group V	Lidocaine hydrochloride (without adrenaline) with 17% EDTA liquid
Group VI	Lidocaine hydrochloride (without adrenaline) with 2% chlorhexidine
Group VII (Control)	Lidocaine hydrochloride (with adrenaline) with deionized water
Group VIII (Control)	Lidocaine hydrochloride (without adrenaline) with deionized water

To 1 ml of lidocaine hydrochloride (with and without adrenaline) in a vial, 1 ml of the corresponding root canal irrigant/de-ionized water was added for each of the 10 samples in all the 8 groups. The vials were left undisturbed and observations were made for 2 hours at an interval of 15 minutes.

The samples were noted for any precipitate formation. The observed precipitates were later subjected to ^1H NMR spectroscopic analysis (^1H ^{13}C) at IIT, Chennai.

RESULTS

Color change and precipitate formation

In groups I and IV, a white-colored precipitate was observed in all the samples at the time of mixing the solutions. Later, this precipitate underwent a color change to reddish-brown color after 15 minutes of mixing the solutions [Figures 1a and b].

The groups II, III, V, and VI showed no precipitate formation or color change in any of the samples, until 2 hours. Similarly, the control groups VII and VIII also showed no precipitate formation or color change in all the samples.

Analysis with nuclear magnetic resonance spectroscopy

NMR analysis of the precipitate showed peaks at 6.8 and 7.1 ppm corresponding to the aromatic ring protons. A peak at 2.2 ppm corresponded to the methylene protons adjacent to the guanidine nitrogen

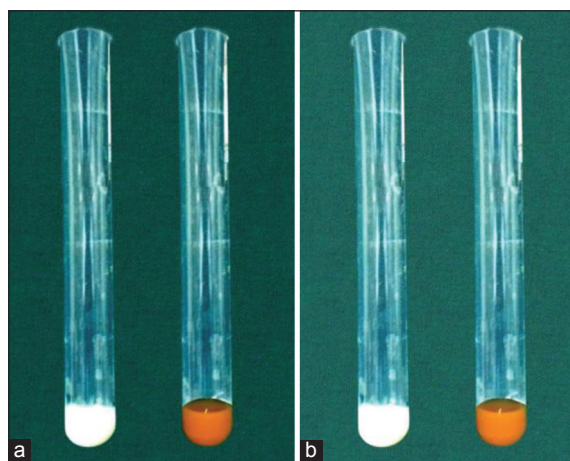


Figure 1: (a) GROUP-I (Lidocaine hydrochloride with adrenaline and 3% NaOCl) White precipitate formed immediately on mixing the solutions and color change observed after 15 minutes (b) GROUP-IV (Lidocaine hydrochloride without adrenaline and 3% NaOCl) White precipitate formed immediately on mixing the solutions and color change observed after 15 minutes

which was related to the characteristic NMR spectra of 2,6-xylylidine, an aromatic amine [Figure 2].

Characterization of the precipitate with diazotation test

The precipitate that was observed was found to be 2,6-xylylidine, an aromatic amine which was confirmed using diazotation test. In diazotation test, addition of nitrous acid/HCl to the precipitate resulted in the formation of diazonium salts. This confirmed the presence of an aromatic amine group characterizing the precipitate.

DISCUSSION

In accordance with evidence-based research, the most widely used root canal irrigants such as, NaOCl, EDTA, CHX are always a combination of two or more solutions.^[14] In an earlier study, Basrani *et al.*, reported that the interaction between NaOCl and CHX resulted in the formation of para-chloroaniline precipitate,^[10] which is a known carcinogen. Rasimick *et al.*, reported the formation of a highly insoluble pink powdery precipitate when 17% EDTA and 1% CHX were mixed, but this was found to be non-toxic.^[15] Ballal *et al.*, based on their study, concluded that maleic acid did not form any precipitate when mixed with NaOCl.^[16]

Several techniques have been utilized for the purpose of studying the interaction between two or more compounds. Techniques such as, Gas Chromatography-Mass Spectrometry, X-ray Photon Spectroscopy (XPS), and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS), High Performance Liquid

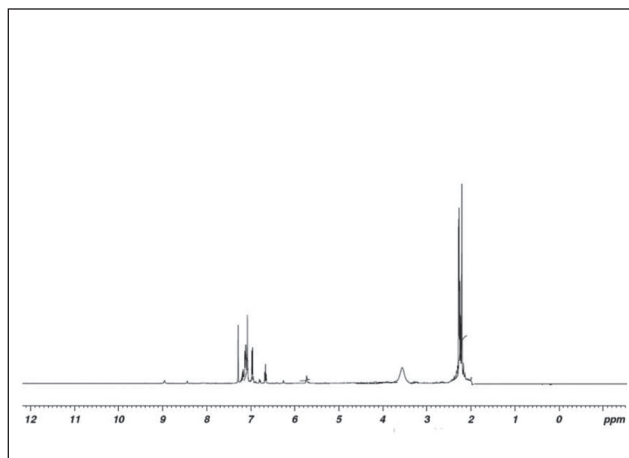


Figure 2: NMR spectra of the precipitate characteristic of 2,6-xylylidine

Chromatography (HPLC), and NMR have been used in the previous studies.^[11,15,17,18]

Time-of-flight secondary ion mass spectrometry was limited in determining the quantity of precipitate formed among various mixtures. Thomas and Sem had argued that mass spectrometry might not be a reliable method for determining the presence of degradation products because it relies on gas phase ionization, which can fragment molecules.^[18] In contrast, NMR spectroscopy analyses molecules in a non-invasive and non-destructive manner.

NMR spectroscopy is one of the principal techniques used to structurally characterize molecules based on chemical shift values and couplings between atoms. Here even the most fragile bonds remain intact. If a molecule is expected to be present in solution, then pure form of the expected molecule can be added to the sample, and the resulting peaks will appear with the same pattern (singlet, doublet, triplet, etc) at the same chemical shift. If a peak does not share the same pattern and chemical shift, then the corresponding molecule is not present in the mixture.^[18]

In the present study, there was no precipitate formed in group II, group III, group V, group VI, and in the control groups VII and VIII. Even though, no precipitate was formed on mixing lidocaine hydrochloride with EDTA and CHX, it is still unclear as to whether lidocaine will actually interfere with the activity of these irrigants.

The reason attributed to the formation of the 2,6-xylylidine compound was due to the acid hydrolytic reaction between lidocaine hydrochloride and NaOCl. When NaOCl interacts with local anesthetic solution (i.e., lidocaine hydrochloride with and without adrenaline), it liberates hypochlorous acid that combines with carbon atoms present in the lidocaine hydrochloride, resulting in the disruption of the molecule with subsequent cleavage of the double bond. On further hydrolysis, 2,6-xylylidine precipitate was formed.

The final precipitate that was formed is an aromatic amine with a benzene ring. Diazotation test confirmed the presence of an aromatic amine in the precipitate. Here, the nitrosation of the precipitate with nitrous acid (generated *in situ* from sodium nitrate and a strong acid, such as hydrochloric acid or sulfuric acid) leads to formation of diazonium salts which takes place in the presence of an aromatic amine, i.e., aniline in the precipitate.^[19] Diazotation test was

positive for the precipitate formed in our study, which confirms the presence of an aromatic amine group.^[1] H-NMR spectrum assigned for aromatic ring protons showed peaks at 6.8 and 7.1 ppm. A peak at 2.2 ppm corresponded to the methylene protons adjacent to the guanidine nitrogen which corresponded to the characteristic NMR spectra of 2,6-xylylidine, which is a known toxic compound.

The clinical significance of 2,6-xylylidine precipitate is related to its toxicity. A previous toxicology study in animals related 2,6-xylylidine compound to the occurrence of carcinomas or adenocarcinomas.^[20]

Although Birchfield *et al.*, in an earlier study, reported that the anesthesia effect of the intra-pulpal injection was due to the back-pressure of the solution (independent on the type of solution), the use of lidocaine hydrochloride for obtaining profound pulpal anesthesia is still prevalent in clinical practice.^[21] Hence, the immediate use of NaOCl following intra-pulpal anesthesia with lidocaine hydrochloride should be avoided to prevent toxic precipitate formation. Separate syringes should be used for administering lidocaine hydrochloride and sodium hypochlorite to avoid such potential interactions.

Mortenson *et al.*, in a study, concluded that least amount of PCA was formed when intermediate flushes of citric acid was used between NaOCl and CHX.^[22] Similarly, Rossi-Fedele *et al.*, suggested intermediate flushing out of NaOCl with saline, water or alcohol prior to the use of CHX to prevent the toxic interactions between these two irrigants.^[23] Hence, following the administration of intra-pulpal anesthesia, flushing out the residual lidocaine hydrochloride with saline, prior to the use of NaOCl might prevent the formation of 2,6-xylylidine precipitate, but this needs to be evaluated. Further investigations are necessary to determine the possible effects of 2,6-xylylidine on dental and periapical tissues. The threshold required to cause hazardous damage in humans with the observed precipitate is still unclear. Hence, the use of combination of sodium hypochlorite and lidocaine hydrochloride should be avoided completely until further studies prove its effect.

CONCLUSION

Within the limitations of this *in vitro* study, it can be concluded that:

- Interaction between lidocaine hydrochloride (with and without adrenaline) and NaOCl resulted in the

formation of a toxic precipitate, 2,6-xylydine, a known carcinogen.

- Interaction between lidocaine hydrochloride (with and without adrenaline) and EDTA or CHX did not result in any precipitate formation.

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