

Comparative evaluation of free active chlorine content of Sodium Hypochlorite under the influence of Lignocaine Hydrochloride, Lignocaine Hydrochloride with adrenaline and Bupivacaine hydrochloride - An In-Vitro study.

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Citation of this Article: Dr. Kunhappan Sanjeev, Dr. Sial Shruti, Dr. Jain Deepti, Dr. Kridutt Vaibhav, Dr. Ratre Shweta, Dr. Siddiqui Taha, "Comparative evaluation of free active chlorine content of Sodium Hypochlorite under the influence of Lignocaine Hydrochloride, Lignocaine Hydrochloride with adrenaline and Bupivacaine hydrochloride - An In-Vitro study", IJDSIR- December - 2022, Vol. – 5, Issue - 6, P. No.96– 102.

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Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Aim: The interaction between irrigants and local anaesthetic (LA) agent becomes inevitable in conditions requiring intrapulpal anaesthesia. Action of LA agent on irrigants solution can alter the properties of the latter. Thus, the present study was undertaken to study the effect of Lignocaine hydrochloride with or without adrenaline and bupivacaine (commonly used LA agents) on the free active chlorine (FAC) concentration of sodium hypochlorite (commonly used irrigants).

Materials and Method: 5.25% w/v of sodium hypochlorite served as control. % Lignocaine hydrochloride containing 1:80.000 adrenaline, 2% Lignocaine hydrochloride without adrenaline and 0.5%

Bupivacaine hydrochloride were the test material. The control and experimental materials were taken in a vial in a ratio of 1:1 and iodometric titration was used to determine FAC. It was repeated five times and average was calculated. One-way anova was performed to obtain difference between the groups.

Results: The mean FAC values were calculated for control and experimental groups. There was significant reduction in FAC concentration from control to experimental group. The experimental groups also showed significant difference amongst each other. The minimum FAC was seen with bupivacaine followed by lignocaine with adrenaline and lignocaine without adrenaline.

Conclusion: The mixing of sodium hypochlorite with LA agents can significantly reduce the active chlorine content which is responsible for chemical action of the former. Thus, the measures should be employed to reduce the interaction as much as possible.

Keywords: Bupivacaine, Free active chlorine, Lignocaine hydrochloride, Sodium hypochlorite

Introduction

Endodontic treatment can be painful at times. This can be attributed to numerous reasons such as presence of acidic environment due to abscess, inactivity of nerve blocks or apical extrusion of debris. To reduce fear, discomfort and anxiety; adequate anaesthesia has to be obtained. The method of achieving anaesthesia which is routinely used is blocking the particular nerve which is supplying the area of interest. There are other techniques also such as infiltration, intra-osseous, inter-septal or intra ligament ARY deposition causing numbing of local area. The most common nerve block for mandibular teeth is Inferior alveolar nerve block, for maxillary teeth, posterior superior alveolar nerve; infraorbital as well as incisive nerve blocks are prevalent. Studies have shown that the success rate of these nerve blocks are fluctuating despite of the proper techniques applied.^[1]

A supplemental injection is administered at times the patient is feeling the “hot tooth” which is intrapulpal anaesthesia. This involves injecting the local anaesthetic (LA) solution directly into the pulp chamber with a positive pressure in an attempt to restore the anaesthesia, while removing the debris out of the canal.^[2] This procedure is usually followed by irrigating the canal with sodium hypochlorite (in concentrations between 0.5% to 5.25%).^[3] However the two fluids in such an enclosed chamber will have an effect on each other. It has been reported that when sodium hypochlorite (NaOCl) interacted with lignocaine with or without adrenaline

forms a carcinogenic precipitate, 2,6-xylydine.^[4] Moreover, interaction of free active chlorine (FAC) which is responsible for the cleansing action of NaOCl by dissolution of debris is also affected by presence of LA molecules in the pulp chamber.^[5]

There are many LA solutions commercially available. Lignocaine is considered as gold standard to evaluate the effect and safety of other LA agents. It has anti-nociceptive, anti-inflammatory and immune-modulating properties best suited for aesthetic and antiarrhythmic benefits.^[6] As it has vasodilating properties, it can be easily absorbed in the blood stream thus diminishing the effect along with shorter duration of action. To enhance this adrenaline solution is added to the LA solution so as to delay the absorption in the body along with increasing the time of action in the tissues. This is attained by vasoconstricting action of adrenaline.^[7] An aniline solution, Bupivacaine has gained interest in terms of its achieving longer-term anaesthesia. It has two isomeric forms, which act on selective nerve fibers to halt pain transmission.^[8] It can be a potent intrapulpal anaesthetic agent.

Hence the present study was under taken to evaluate and compare the free active chlorine content of sodium hypochlorite under the influence of lignocaine hydrochloride, lignocaine hydrochloride with adrenaline and Bupivacaine hydrochloride.

Materials and Method

The study was conducted in a laboratory set up. The root canal irrigants and anaesthetic solutions were taken in vials and dilution were done as planned. The test sample consisted of 5.25% w/v of NaOCl (Vishal® Dentocare Pvt Ltd, Ahmedabad), 2% Lignocaine hydrochloride containing 1:80,000 adrenaline (RBS Pharmaceuticals), 2% Lignocaine hydrochloride without adrenaline (RBS Pharmaceuticals) and 0.5% Bupivacaine hydrochloride

(NEON, Chennai). The control group (Group I) consisted 10 ml of NaOCl which was undiluted. It served as effective concentrate for the test groups. This group (Group I) indicated the actual FAC content of undiluted NaOCl. The test groups were three: Group II - NaOCl + Lignocaine, Group III - NaOCl + Lignocaine with adrenaline and Group IV - NaOCl + Bupivacaine. The experimental solution was made by diluting the NaOCl and anaesthetic solution in 1:1 ratio (i.e., 5 ml: 5 ml). The FAC was calculated by iodometric titration method which was repeated five times for each test group. All the groups (test and control) were evaluated and average was obtained.^[9]

Titration procedure: An iodometric titration process was performed for all the groups and active chlorine concentration (C_{FAC}) was attained. A 10mL of the sample solution was pipetted into a 100 mL flask from each group (V_s). These analytic solutions were then treated with iodide ion (I^-) (Chenchems, Chennai, India). This iodide ion is a weak reducing agent, which undergoes oxidation to elemental iodine (I_2). This elemental iodine combines with any excess iodide present to produce I_3^- . This ion, I_3^- forms a coloured complex with starch giving a bluish black appearance. Then, it was titrated with 0.1 mol/L concentration (C_T) of standardized thiosulphate ($S_2O_3^{2-}$) (Chenchems, Chennai, India) till the bluish black solution became colourless and the total volume of thiosulphate (V_T) added into it was noted. This colour change occurred due to the conversion of elemental iodine back to iodide ion (I^-). These chemical processes are generally redox reactions and hence, this analysis is referred to as a redox titration.^[9]

The concentration of FAC was then calculated using the following formula

$$C_{FAC} = V_T \times C_T \times M_{Cl} \text{ (g/mol) VS}$$

Where, V_T =Volume of the standardised thiosulfate used for the titration (in mL), C_T =Concentration of standardized thiosulfate (0.1 mol), V_s =Volume of respective experimental and control solutions (10mL) and M_{Cl} =Molar atomic mass of chlorine (35.4527 g/mol). The study was conducted in Department of Chemistry, Integral University, Kursi Road Lucknow.

Statistical Analysis

One-way Anova was performed along with post hoc Tukey using IBMSPSS Statistics Version 22.0 (IBM Corp, Armonk, NY, USA).the mean FAC was compared amongst the various groups. The significance was considered when $p < 0.05$.

Result

The mean FAC values for all the groups are shown in table 1 and graph 1. One-way Anova showed a significant difference between the groups. The control group was taken as standard for the mean FAC which showed maximum chlorine content which reduced arithmetically in the experimental groups. The minimum FAC was obtained with Group IV which was 0.31, there was significant difference obtained in terms of mean between control group and the experimental groups (table 2). Also, there was a significant difference amongst the three experimental groups when compared to each other (table 3). When Group II and Group III were compared there is more reduction of FAC in NaOCl + Lignocaine with adrenaline solution, and minimum FAC was obtained with bupivacaine.

Discussion

The undisputable method of success in endodontic is complete disinfection of root canal system. The instruments mainly are confined in the main canal and accessory canals are often unapproachable. The efficient use of irrigants can lead to complete dissolution of

microbial debris as well as remnants of bodily tissues in these canals, which, if present, can lead to failure of endodontic therapy in many aspects. Borin et al have concluded that irrigants promote hydraulic circulation in the canal resulting in effective dissolution of organic contents of canal.^[10] The judicious and unaltered use of irrigating solution is thus a prime requisite for endodontics.

Sodium hypochlorite (NaOCl) is widely used endodontic irrigants and its use dates back to 1920s.^[11] It is available commercially in various concentrations ranging from 0.5% to 5%. The active ingredient which brings about the tissue dissolution, removal of predentin and exerts antimicrobial action is the concentration of available chlorine or FAC.^[12] This FAC is the unbound active ingredient in the form of hypochlorous acid (HOCl) and hypochlorite ions (OCl⁻).^[13] This liberation of FAC is largely affected by the presence of another liquid solution in the canal. There are numerous irrigants which are combined in the root canal so as to bring about the desired effect such as EDTA, CHX or LA solution. The supplemental injection which is administered in the form of intrapulpal anaesthesia is basically done to reduce the pain of patient which is persisting even after nerve block. The major advantage of this technique is that when given with adequate pressure it can reduce the pain quite effectively and with immediate onset.^[14] Thus, the interaction of LA solution with NaOCl in the confined canal can affect its properties.

In the present study, three different composition amides local anaesthetics are used to study their effects on FAC when admixed with NaOCl. The pure NaOCl which served as control was mixed with this LA solution in the ratio of 1:1. The trituration process was similar to that of Gurcharan et al,^[9] the results obtained are also coinciding

with that of authors. The results have shown that there was a significant reduction in terms of FAC. The mixture of NaOCl and bupivacaine has shown a minimum release of active chlorine. The possible mechanism of it could be the low pH of bupivacaine which can make NaOCl unstable. Pradhan et al quoted that as the pH decreases from 11 to 7, decomposition also increases and is highest at pH 7.^[15] Thus it can lead to low release of active chlorine.

Lignocaine when comes in contact with NaOCl undergoes an acid hydrolytic reaction. This results in release of hypochlorous acid which combines with carbon atoms present in LA molecule. This leads to disruption with subsequent cleavage of the double bond of hypochlorous acid.^[1] This leads to formation of precipitate. This is the possible mechanism of reduced active chlorine in NaOCl with lignocaine group.^[9] Another effect of the same is that the precipitate which is formed act as a barrier for penetration of intra-canal irrigants/medicaments. This compromises the canal seal too. If the precipitate is not completely removed from the canal walls, coronal seal of post endodontic restoration is also affected.^[16] This can be avoided if there is adequate rinsing of the root canal after administration of intrapulpal anaesthesia. Also, separate syringes should be used for both the solutions.^[4] Shetty et al have concluded that use of ultrasonic instruments are required for efficient removal of the, 2,6-xylydine formed on the root surface as a result of this interaction.^[17]

The concentration of FAC is further reduced in NaOCl + lignocaine hydrochloride with adrenaline group. This result can again be attributed to decrease in pH by addition of vasoconstrictor. The results obtained are similar to that of Gurcharan et al^[9] and Vidhya et al.^[4] The clinical relevance of this study lies in the result

obtained that the presence of vasoconstrictor in a LA solution will reduce the FAC of NaOCl. The use long term anaesthesia bupivacaine prove to be further deteriorating in chemo mechanical efficiency of NaOCl. Saravana Karthikeyan et al have advised to use normal saline instead of intrapulpal anaesthesia since the principal behind the same is backpressure of the liquid used.^[18]

The intrapulpal anaesthesia becomes necessary since the success rates of nerve blocks can be varying. The LA solution present in the canal is bound to have some effect on the irrigating solutions which are used. Thus, the present study was done in an attempt to analyse the effect of three different types of LA solution when mixed with sodium hypochlorite. To the best of authors' knowledge, this is the first study to evaluate effect of bupivacaine with NaOCl. The results indicate the mixture can adversely affect dissolution properties of NaOCl. This an in vitro study, thus result obtained may differ from the in vivo set-up. Trituration method was used in the present study. The results should be confirmed by other methods such as nuclear magnetic resonance spectroscopy (NMR), liquid chromatography and tandem mass chromatography and diazotation test. Similar studies should be undertaken with use of more than one method simulating natural conditions are warranted.

Conclusion

Within the limitations of this study, it can be stated that 1) mixture of sodium hypochlorite with lignocaine with or without adrenaline will adversely affect the chemical properties of it. 2) The use of bupivacaine should be avoided despite of it having long term anaesthesia as it showed minimum release of active chlorine which is crucial for the action of NaOCl. 3) Special care should

be done when intrapulpal anaesthesia is administered to clear the canal before using root canal irrigants.

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Table Legends

Table 1: Table showing descriptive statistics of Free Active Chlorine content obtained from various groups.

Free Active Chlorine Content	N	Mean	Std. Deviation	Minimum	Maximum
Group I	5	3.8300	.18668	3.58	4.07
Group II	5	1.0360	.03912	.99	1.09
Group III	5	.6940	.05128	.63	.77
Group IV	5	.3120	.05541	.24	.38
Total	20	1.4680	1.42670	.24	4.07

Table 2: Table showing mean FAC values of various groups.

One way ANOVA					
Free Active Chlorine Content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	38.505	3	12.835	1.220E3	.001*
Within Groups	.168	16	.011		
Total	38.674	19			

Table 3: Table showing comparison of mean FAC values using Post hoc Tukey.

Tukey HSD						
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group I	Group II	2.79400*	.06487	.001*	2.6084	2.9796
	Group III	3.13600*	.06487	.001*	2.9504	3.3216
	Group IV	3.51800*	.06487	.001*	3.3324	3.7036
Group II	Group I	-2.79400*	.06487	.001*	-2.9796	-2.6084
	Group III	.34200*	.06487	.001*	.1564	.5276
	Group IV	.72400*	.06487	.001*	.5384	.9096
Group III	Group I	-3.13600*	.06487	.001*	-3.3216	-2.9504
	Group II	-.34200*	.06487	.001*	-.5276	-.1564
	Group IV	.38200*	.06487	.001*	.1964	.5676
Group IV	Group I	-3.51800*	.06487	.001*	-3.7036	-3.3324
	Group II	-.72400*	.06487	.001*	-.9096	-.5384
	Group III	-.38200*	.06487	.001*	-.5676	-.1964
*. The mean difference is significant at the 0.05 level.						

Graph 1: Graph showing mean Free Active Chlorine content obtained from various groups.

