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Efficacy of Chlorine Dioxide as an Endodontic Irrigant for Pulp Dissolution *in vitro*

Eficacia del Dióxido de Cloro como Irrigante Endodóntico para la Disolución Pulpar *in vitro*

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ABSTRACT: Determine the efficacy of 5 % chlorine dioxide as an endodontic irrigant for pulp dissolution. Thirty five samples of human dental pulp were obtained, previously weighed and immersed in three solutions= 5 % ClO_2, 5.25 % NaOCl and saline (control group), for 10 minutes at 32 ºC; they were dried and reweighed. Then the weight loss was compared to the original weight and analyzed statistically. 5.25 % NaOCl and 5 % ClO_2 dissolved the dental pulp samples more effectively than saline (p> 0.001). No statistically significant difference was found between the tissue dissolving properties of 5.25 % NaOCl and 5 % ClO_2 (p=0.893). 5 % ClO_2 is effective in dissolving human dental pulp tissue.

KEY WORDS: pulp dissolution, chlorine dioxide, endodontic irrigants, sodium hypochlorite.

INTRODUCTION

An endodontic irrigant must have these main properties: antimicrobial activity, solubility in water, low toxicity to periradicular tissues, and the ability to dissolve tissues. Irrigation is a very important step because it can reach inaccessible areas that are not cleaned directly with the instruments. The dissolution of the pulp tissue is a desirable property of any irrigating solution since it improves the cleaning of the root canal (Niewierowski *et al*., 2015). Sodium hypochlorite (NaOCl) is the most frequently used irrigant in endodontic therapy due to a great capacity of tissue dissolution and an extensive antimicrobial activity. However, one disadvantage is its high cytotoxicity on periradicular tissues and oral mucosa. In addition to, NaOCl is reported to form chlorinated hydrocarbons known to be carcinogenic (Cobankara *et al*., 2010; Singh *et al*., 2012).

Chlorine dioxide (ClO_2) may be a potential alternative for endodontic treatments as a root canal irrigant, as it shows antimicrobial properties, low toxicity, and the non-production of by-products. However, there is minimal information on the percentage of chlorine dioxide suitable for the dissolution of the pulp tissue (Singh *et al*., 2012). Previous studies used different concentrations of ClO_2 capable of dissolving tissues where it was compared with NaOCl (Cobankara *et al*., 2010; Singh *et al*., 2012; Herczegh *et al*., 2013; Baisawala *et al*., 2018). NaOCl was found to be more effective than 5 % ClO_2 at room temperature and acidic pH in a couple of investigations (Cobankara *et al*., 2010; Taneja *et al*., 2014). It has also been reported that 13.8 % ClO_2 at 37 ºC and alkaline pH was more effective than NaOCl in dissolving tissue (Baisawala *et al*., 2018). However, in other studies, no statistically significant differences were found between the tissue dissolution properties of NaOCl and 13.8 % ClO_2 at room temperature and alkaline pH (Singh *et al*., 2012). The results of these works are contradictory. Therefore, we decided to clarify the dissolution effect of ClO_2 in a stable presentation that is 5 %, warm temperature and at an alkaline pH. The main objective of this study was to determine the efficacy of 5 % chlorine dioxide for the dissolution of human pulp.

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MATERIAL AND METHOD

Dental pulp was obtained from extracted teeth. The teeth were obtained from patients treated at the Dental Center of the University of San Martin de Porres. This work followed the 1975 Declaration of Helsinki. Ethical approval of the study (dated September 17, 2019 and numbered 025-2019) was obtained from Research Ethics Committee of the Faculty of Dentistry at the University of San Martin de Porres. After the extracted teeth were placed in saline solution and refrigerated for 24 hours, the dental pulp was removed using a curette and forceps (Fig. 1). Then, it was washed in distilled water to remove excess blood, and dried with paper (Fig. 2). After this, it was refrigerated for 30 min to help section the pulp tissues. Each sample had a standard weight of 15 mg-25 mg (Fig. 3). The initial weights of each sample were measured with a precision digital analytical scale. Throughout the experiment, the tissues were handled only with forceps to avoid contamination and errors in weight.

The samples were individually coded and randomly divided into 3 groups, corresponding to the type of solution to be used in each group: 5 % ClO_2 solution (Bac Food, Lima), 5.25 % NaOCl (All Clean, Lima) and saline solution (Fig. 4). pH measurements of all study solutions were performed. After the test solutions were placed in the tubes containing the tissue samples, the tubes were placed in a vibrator and at 32° C for 10 min. In this way, all tissue is fully exposed to irrigant solution. The tissue samples were then removed from the solutions, washed with distilled water to remove any suspended tissue debris, dried with sterile blotting paper and weighed again. All samples before and after exposure to the test solution were weighed by a single investigator. The percentage of tissue weight loss or gain was obtained.

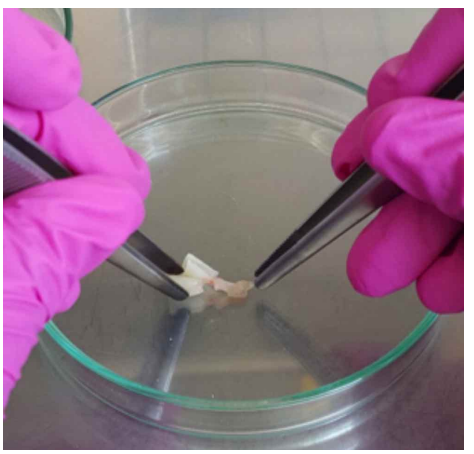

Fig. 1. The hard tissues are separated from the pulp tissue.

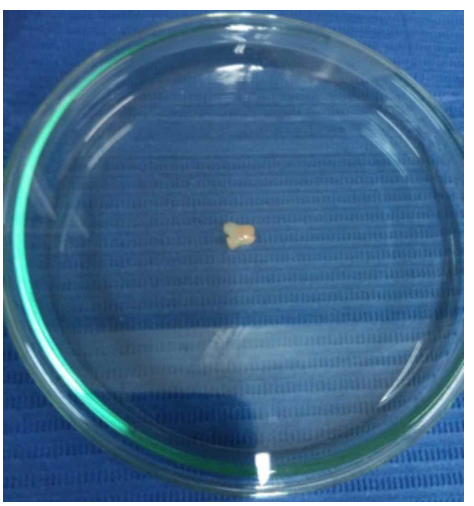

Fig. 2. The pulp tissue.

Fig. 3. The sample showing a weight of 15 mg.

Fig. 4. Samples divided into three groups.

Descriptive statistical analyzes were performed with measures of central tendency. For the statistical comparison of the three groups, the Kruskal Wallis test was used, in which the data were processed at the 95 % confidence level. The paired comparison was also analyzed with the Bonferroni Correction Post-Hoc test.

RESULTS

Percentages of weight change mean values and standard deviation of the dental pulp after exposure to irrigant solutions are shown in table I. Statistically significant differences are observed when comparing the 5 % chlorine dioxide and saline solution (negative control group) ($p < 0.001$) and when comparing 5.25 % sodium hypochlorite (positive control group) and the saline solution ($p < 0.001$). The comparison of chlorine dioxide and 5.25 % sodium hypochlorite does not show statistically significant differences ($p = 0.983$) at a 95 % confidence level.

Different letters indicate statistically significant differences ($p<0.001$). Equal letters indicate that there are no statistically significant differences ($p=0.983$) (Bonferroni correction post-hoc Tests).

Table I. Weight change and standard deviation (SD) in percentage weight of human pulp tissue fragments after immersion in irrigating solutions

Irrigant Solutions	N	Weight change (%) ± SD
5 % Chlorine Dioxide	13	- 98.44 ± 3.26[a]
5.25 % Sodium Hypochlorite	13	- 98.45 ± 3.19[a]
Saline Solution	9	- 3.62 ± 14.53[a]

DISCUSSION

The dissolution of the pulp tissue is one of the main requirements of an endodontic irrigant (Niewierowski *et al*., 2015; Cullen *et al*., 2015). The use of some disinfecting agents for endodontic irrigation has recently been proposed, among them ClO_2, but its potential as a solvent for pulp tissue has not yet been fully explored. The objective of this research was to determine the efficacy of 5 % ClO_2 as an endodontic irrigant for the dissolution of human dental pulp.

Narayanan *et al*. (2017) showed that the dissolution of the tissue depended on important factors such as the type of tissue, the amount of organic matter in relation to the amount of irrigant, exposure time, concentration, pH of the solution and temperature. In the present study, human pulp tissue was used, and each sample had similar weight (average 20.17 mg). We also considered a constant agitation of 1000 rpm per minute to simulate fluid movement produced during the instrumentation, and the same volume of irrigant for all samples.

Cobankara *et al*. (2010) and Baisawala *et al*. (2018) immersed the pulp samples in the solutions for 20 minutes with a replacement every 2 minutes, time for the first phase of the NaOCl to last when it is consumed and becomes unstable (Basrani & Haapasalo, 2012). However, there are other studies that did not perform a replacement (Singh *et al*., 2012; Jain *et al*., 2015). During the pilot tests of this study, immersing the samples in 5.25 % NaOCl at 15 minutes completely dissolved them. Therefore, a fixed period of 10 minutes without replacement was used. However, it is still necessary to determine the optimal time that the irrigant should remain in the dentinal tubules (Basrani & Haapasalo, 2012).

The minimum concentration of ClO_2 to dissolve the pulp tissue is unknown. Previous studies used concentrations of 5 %, 13 % and 13.8 % (Cobankara *et al*., 2010; Singh *et al*., 2012; Taneja *et al*., 2014; Baisawala *et al*., 2018) We acquired the available maximum concentration (10 %), but this solution was unstable. It is reported that chlorine dioxide is unstable above the 10 % concentration ([ClO2]/[air]) (Dobson & Cary, 2002). Therefore, we used ClO_2 at a stabilized concentration of 5 %.

The pH of the irrigant solution could affect the dissolution of the tissue. Previous reports have shown an inverse relationship between the pH of a solution and the time it takes for the solution to dissolve tissue. Singh *et al*. (2012) showed that ClO_2 at pH 4.67 was less effective than NaOCl used at pH 12 in dissolving pulp tissue. In contrast, Cobankara *et al*. (2010) and Baisawala *et al*. (2018) by increasing the pH of ClO_2 to 12 with sodium hydroxide, they found good pulp dissolution equivalent to the efficacy of NaOCl. However, Deininger *et al*. (2012) warns the negative effects when calibrating the pH, such as the formation of other compounds or loss of the biocidal effect and recommends the use of the manufacturing pH. Therefore, in the present study, the manufacturing pH of the ClO_2 and NaOCl solutions were maintained at 11.30 and at 12.39 respectively.

Previous studies in which solutions were used at room temperature resulted in low effectiveness of ClO_2 (Singh *et al*., 2012; Taneja *et al*., 2014; Jain *et al*., 2015). Other researchers conclude that heating irrigant solutions such as NaOCl and ClO_2 increases their ability to dissolve organic matter (Dumitriu *et al*., 2015; Baisawala *et al*., 2018). In the present study, the solutions were heated to 32 ºC to adjust to the temperature determined in the root canal (De Hemptinne *et al*., 2015).

Both solutions 5 % chlorine dioxide and 5.25 % sodium hypochlorite showed a significant pulp tissue dissolution compared to saline solution (control group). As expected, the samples immersed in control group showed very little percentage of weight loss. This finding is in agreement with previous studies in which dissolution was more effective in samples immersed in ClO_2 and NaOCl solutions compared to saline solution (Singh *et al*., 2012). In this study, it was observed that the percentage of weight loss of the samples immersed in 5 % ClO_2 and 5.25 % NaOCl did not show statistically significant differences. This finding coincides with previously reported results showing that ClO_2 is just as effective as NaOCl (Cobankara *et al*., 2010). On the other hand, Baisawala *et al*. (2018) concluded that 13.8 % ClO_2 was more effective than 3 % NaOCl. This contrast may be attributed to the difference in temperature and pH used. Baisawala *et al*. (2018) heated the solutions up to 37 ºC and worked with alkaline pH. On the other hand, there are reports showing that ClO_2 at 5 %, 13 %, 13.8 % is less effective compared to NaOCl from 2.5 % to 5.25 % (Singh *et al*., 2012; Taneja *et al*., 2014; Jain *et al*., 2015). This contrast probably occurred because the solutions were prepared at room temperature and the ClO_2 was used at an acidic pH in these investigations. Thus, it is necessary to calibrate the conditions to improve the dissolution effect of ClO_2 and NaOCl (Singh *et al*., 2012; Narayanan *et al*., 2017; Basaiwala *et al*., 2018).

Consequently, this research increased theoretical knowledge of the properties and efficacy of chlorine dioxide at a stable minimum concentration. This solution could be applied in clinical trials, as a new alternative for irrigation in endodontic therapies. Its potential use would be beneficial for both the patient and the operator. Chlorine dioxide has adequate properties, its effectiveness in pulp dissolution and the most relevant being its low toxicity reducing accidents caused by other irrigants such as NaOCl.

CONCLUSION

It was concluded that after immersing the dental pulp in the irrigation solutions, 5 % chlorine dioxide is effective as 5.25 % sodium hypochlorite for dissolving human dental pulp *in vitro*.

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RESUMEN: El objetivo de este estudio fue determinar la eficacia del dióxido de cloro al 5 % como irrigante endodóntico para la disolución pulpar. Se obtuvieron 35 muestras de pulpa dental humana, se pesaron previamente y se sumergieron en tres soluciones= 5 % ClO2, 5.25 % NaOCl y suero fisiológico (grupo control), durante 10 minutos a 32 ºC; se secaron y se pesaron de nuevo. Luego se comparó la pérdida de peso del peso original y se analizó estadísticamente. NaOCl al 5.25 % y ClO_2 al 5 % disolvieron las muestras de pulpa dental con más eficacia que el suero fisiológico (p> 0.001). No se encontró diferencias estadísticamente significativas entre las propiedades de disolución de tejido de NaOCl al 5.25 % y ClO_2 al 5 % (p=0.893) ClO_2 al 5 % es eficaz para disolver tejido de pulpa dental humana.

PALABRAS CLAVE: disolución pulpar, dióxido de cloro, irrigantes endodónticos, hipoclorito de sodio.

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