Solvent effect of sodium hypochlorite solutions commercialized in Pelotas (Brazil) on bovine dental pulp

Milena Goveia Mathies **HASSE**¹ Juliana Freitas **GONÇALVES**¹ Julio Cesar Emboava **SPANO**² Luiz Fernando Machado **SILVEIRA**² Josué **MARTOS**² Eduardo Luiz **BARBIN**²

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ABSTRACT

Objective: The aim of this study is to evaluate the effect of sodium hypochlorite solvent solutions on bovine pulp tissue by analyzing products commercialized in Pelotas/RS, Brazil. **Methods:** The following substances were tested: Dakin's solution, Milton's solution, Labarraque's solution and double-strength chlorinated soda (5% sodium hypochlorite). Prescriptions were given out by compounding pharmacies and solutions were bought in dental supply stores. Four hypochlorite-based bleaches (Qboa, Clorofina and Brilhante), three bought at ordinary grocery stores and one solution prepared in a compounding pharmacy, were also evaluated. To test tissue dissolution, a peristaltic

pump with 15 ml sodium hypochlorite was used. Bovine pulp fragments, previously sectioned into 10 mm slices from their central portion, were inserted into the pump. Pulp dissolution data were tested for normality by means of Anderson-Darling test which directed towards parametric and nonparametric tests. **Results:** 5% sodium hypochlorite proved to have the greatest pulp tissue dissolving ability. Dakin's solution proved the lowest while 2.5% and 2.0 to 2.5% products had intermediate bovine pulp dissolving ability. The ability to dissolve bovine pulp tissue was not observed in all samples tested.

Keywords: Dental pulp. Sodium hypochlorite. Dissolution.

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¹Multiprofessional Residency in Health, Children's Health, Federal University of Pelotas (UFPel). ²Professor, Department of Semiology and Clinics, School of Dentistry — Federal University of Pelotas (UFPel). » The authors report no commercial, proprietary or financial interest in the products or companies described in this article.

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Contact address: Eduardo Luiz Barbin Faculdade de Odontologia da Universidade Federal de Pelotas – Rua Gonçalves Chaves, 457 – Pelotas/RS – Brazil – CEP: 96.015-560 E-mail: eduardo.barbin@ufpel.edu.br

Introduction

Chemo-mechanical preparation aims at promoting cleaning and shaping of the root canal by means of endodontic instruments, auxiliary chemical substances and irrigation-aspiration processes.¹ Mechanical preparation of the root canal is associated with chemical solutions with antimicrobial properties capable of not only dissolving and removing pulp remnants inside the root canal, but also removing smear layer by means of irrigation, aspiration and flooding processes.^{2,3,4}

Several chemical substances may be used to assist disinfection of the root canal system, namely: halogen compounds (e.g., sodium hypochlorite), synthetic detergents, chelating substances (EDTA), associations (MTAD) and other irrigating solutions such as distilled water, hydrogen peroxide, saline solution and chlorhexidine digluconate.^{4,5}

Sodium hypochlorite, among the aforementioned irrigating solutions, has proved excellent for chemomechanical preparation of the root canal.^{4,6,7} One of its most important features is its tissue dissolving ability. For this reason, sodium hypochlorite is considered the most effective in dissolving pulp tissue.⁷ Sodium hypochlorite causes pulp tissue fragments to be liquefied; thus, facilitating its removal from the root canal by suction.^{1,2,6,8}

Sodium hypochlorite (NaOCl) aqueous solutions obtained by electrolysis have a varying concentration of 10 to 17%. Diluted sodium hypochlorite produces different concentrations of chlorinated solutions used in Endodontics and as hypochlorite-based bleaches. Dissolution of bovine pulp tissue promoted by sodium hypochlorite at concentrations of 0.5, 1.0, 2.5 and 5.0% evinces a directly proportional relationship between pulp dissolution rate and solution concentration.^{2,6,8} Leonardo⁹ observed that the higher the concentration of sodium hypochlorite, the greater its tissue dissolving ability and neutralization of root canal toxicity.

Thus, the aim of this study is to evaluate the effect of sodium hypochlorite solvent solutions on bovine pulp tissue by analyzing products commercialized in Pelotas/RS — Brazil.

Material and Methods

To assess the ability of sodium hypochlorite-based products to dissolve bovine pulp tissue, we initially

used the method described by Spanó.⁸ It consists in using a peristaltic pump that circulates a given volume of solution within a closed system, isolated from the environment, in which a segment of bovine pulp tissue is subjected to the tested solution. The time for complete pulp dissolution is measured by a stopwatch.

The sodium hypochlorite-based commercial products used in the present study were as follows: 0.5% sodium hypochlorite neutralized with boric acid (Dakin's solution), 1.0% sodium hypochlorite stabilized with sodium chloride (Milton's solution), 2.5% sodium hypochlorite (Labarraque's solution or chlorinated soda), 2.0 to 2.5% sodium hypochlorite (sodium hypochlorite-based bleaches) and 5.0% sodium hypochlorite (chlorinated soda).

The origin of the sodium hypochlorite-based products is reported as follows: Iodontosul (Iodontosul, Porto Alegre/RS — Brazil), Asfer (Asfer Chemical Industry, São Paulo/SP — Brazil), Vida Nova compounding pharmacy, Uso Indicado compounding pharmacy, Homeopathus compounding pharmacy and School of Dentistry — UF-Pel. Three commercial brands of sodium hypochloritebased bleach were bought at ordinary grocery stores and another one was acquired at a compounding pharmacy: Qboa (Anhembi Indústrias S.A. Osasco/SP — Brazil), Clorofina (Isnar Coutinho & Filhos Ltda., Pelotas/RS — Brazil), Brilhante (Unilever, São Paulo/SP — Brazil) and Vida Nova compounding pharmacy.

All solutions were stored in a cool environment, protected from sunlight and evaluated shortly after acquisition. Table 1 lists the products used in the present study, as well as their concentration, lot number, date of manufacture and expiration date.

Obtaining and preparing bovine pulps

Immediately after slaughter carried out for meat production for human consumption, (Frigorífico Roloff, Pelotas, Brazil), bovine jaws were collected and extractions performed. The extracted teeth were stored in plastic bags, packed in an insulated box containing ice and transported to the School of Dentistry — UFPel for removal of pulp tissue. Thus, the ethical issues involving bovine pulp no longer exist, since teeth were obtained from animals slaughtered for reasons beyond the scope of our study.

In a clinical/laboratory environment, the root portion of extracted teeth was trapped in a vise (bench vise).

Manufacturer	Concentration	Lot number	Date of manufacture	Expiration date
lodontosul	0.5%	0942	April, 2011	April, 2012
lodontosul	1.0%	0901	March, 2011	March, 2012
lodontosul	2.5%	0948	April, 2011	April, 2012
lodontosul	5.0%	0949	April, 2011	April, 2012
Asfer	0.5%	2334	April, 2011	January, 2012
Asfer	1.0%	4316	July, 2011	October, 2011
Asfer	2.5%	3633	June, 2011	March, 2012
Vida Nova	1.0%		July, 2011	October, 2011
Vida Nova	2.0 to 2.5%		July, 2011	January, 2012
Vida Nova	5.0%		July, 2011	October, 2011
Uso Indicado	5.0%		September, 2011	November, 2011
Homeopathus	2.5%		July, 2011	August, 2011
Qboa	2.0 to 2.5%	L11363	May, 2011	November, 2011
Clorofina	2.0 to 2.5%	LC 11053	June, 2011	November, 2011
Brilhante	2.0 to 2.5%	FFF 091655974	June, 2011	December, 2011

Table 1. Individual characteristics of the products tested.

Tooth fracture was promoted by means of pressure exerted by the vise, followed by removal of pulp tissue. The method preserved pulp tissue integrity in 100% of cases, since the movement produced by the vise jaws was insignificant after dental fracture, thereby avoiding crushing the pulp tissue among dental fragments. Bovine pulps were then washed in saline solution so as to remove potential tooth fragments, wrapped in plastic film (Magipak, Improco Ind. e Com., São Paulo/SP, Brazil), and frozen. We obtained approximately 100 fresh adult bovine pulps of mandibular central incisors.

Before conducting the tests, the pulps were removed from the cooler at -9°C and kept on the bench at room temperature for a period of 90 minutes, the time required for defrost and heat balance. The axis of the central portion of each bovine pulp was cross sectioned with a scalpel blade 15 so as to obtain a 10-mm fragment. Fragment ends were not included for standardization of the outer surface of each pulp fragment.

Prior to the dissolution test, the fragment to be used was weighed on a precision scale (Aaker Solutions Ltda., Porto Alegre, RS, Brazil). Pulp fragments were randomly collected for each test.

Pulp dissolution procedures

All dissolution procedures were performed together under similar conditions (relative humidity and temperature). To test pulp dissolving ability, a polyurethane hose was adapted to the Luer Slip tip of a glass syringe, while the other end of the hose was connected to the inlet of a peristaltic pump. Another hose segment connected the outlet of the peristaltic pump to the inlet port of the barrel of the syringe by means of a rubber stopper perforated at the center.

In this system, 15 mL of the solution to be tested were injected. It circulated through the peristaltic pump and within the closed system with a constant flow of 0.983 milliliters per second. Inside the glass syringe and near the Luer Slip nozzle opening, a nylon net was placed to hold the suspended bovine pulp fragment at this site of the syringe not only to ensure that the pulp fragment was kept immersed in the solution stream (flowing from the Luer Slip tip of the syringe barrel to the nozzle opening), but also to prevent the fragment from entering into the loop of tubing.

A stopwatch was activated at the same time the pulp fragment was immersed into the solution to be tested. The pulp fragment was kept immersed in the circulating solution until it had completely dissolved, at which time the stopwatch was stopped. The time spent for complete dissolution of the pulp was recorded. The point of complete dissolution was established when the pulp fragment was no longer visible to the naked eye.

We opted to use two indicators to assess the ability of sodium hypochlorite-based products to dissolve bovine dental pulp. First, the dissolution rate of the pulp; and second, based on experimental performance, the estimated time required in minutes for complete dissolution of a 20-mg bovine pulp fragment per a volume of 15 mL of NaOCl solution. To calculate the dissolution rate of the pulp, the values of pulp mass, expressed in milligrams (mg), remained bound to the dissolution time expressed in seconds (s). Mass was then divided by time to calculate the pulp dissolution rate expressed in milligrams per second (mg/s).

Based on the premise that pulp dissolution rate indicates the mass of pulp tissue (mg) dissolved per second by a given solution of sodium hypochlorite (15 mL), it is possible to determine the time (s) necessary for dissolving an arbitrary value of pulp mass (20 mg) by means of a simple rule of three. For a better understanding, the period of time expressed in seconds was converted into minutes by dividing the number of seconds by 60. Thus, based on an experimental performance, this procedure allowed us to assess the estimated time required in minutes for complete dissolution of a 20-mg bovine pulp fragment per a volume of 15 mL of NaOCl solution.

Statistical analysis of the difference between the means of the groups assessed according to different designs was determined by means of the statistical software Minitab 14 (Minitab Inc., Pennsylvania, USA). Anderson-Darling normality test directed our study towards parametric tests (ANOVA, Tukey test or t-test) or nonparametric tests (Mann-Whitney, Kruskal-Wallis and Dunnett).

Results

The present study evaluated 16 sodium hypochlorite-based products of which 5 were produced in compounding pharmacies and ten were industrialized. A sample produced at the polyclinic of the School of Dentistry — Federal University of Pelotas (UFPel) was also used. Table 2 shows the dissolution rate of the 12 samples that completed the dissolution process, i.e. in which bovine pulp tissue was completely dissolved.

Four of the 16 products (three from compounding pharmacies and one industrialized) did not complete the bovine pulp tissue dissolution process employed in this study. These products did not show any solvent action, namely: 0.5% NaOCl (Dakin's solution - Asfer), 1.0% NaOCl (Milton's solution - Vida Nova), 5% NaOCl (chlorinated soda - Vida Nova) and 5% NaOCl (chlorinated soda - Homeopathus).

We conducted a comparative analysis of 2.5% NaOCl and 2.0 to 2.5% NaOCl solutions. The group comprising 2.5% NaOCl solutions yielded 15 pulp dissolution rate values (mg/s) for three different products: Asfer (chlorinated soda), Iodontosul (Labarraque's solution) and UFPel (2.5% NaOCl), each one with five results. The group comprising hypochlorite-based bleaches, however, yielded 20 dissolution rate values for tests conducted with four products: Vida Nova, Qboa, Clorofina and Brilhante, each one with five results. The normality test enabled us to conduct the t-test which showed no statistically significant differences (p = 0.964) between the mean dissolution rate of the two pulp samples.

The mean pulp dissolution rates of solutions at different concentrations were also compared considering the following groups: 0.5% NaOCl solution (Dakin's solution); 1.0% NaOCl solution (Milton's solution); 2.5% and 2 to 2.5% NaOCl solutions (chlorinated soda, Labarraque's solution and hypochlorite-based bleaches); and 5.0% NaOCl solution (chlorinated soda). Importantly, for the comparative study conducted in this section, the results of 2.5% NaOCl and NaOCl 2.0 to 2.5%-based products were arranged in one single group.

The group comprising 0.5% NaOCl solutions yielded five pulp dissolution rate values (mg/s) for tests conducted with Iodontosul (Dakin's solution). The group comprising 1.0% NaOCl solutions yielded ten pulp dissolution rate values (mg/s) for tests conducted with two products: Asfer and Iodontosul (Milton's solution). The group comprising hypochlorite-based bleaches and 2.5% NaOCl-based products yielded 35 pulp dissolution rate values (mg/s) for tests conducted with seven products: four hypochlorite-based bleaches (Vida Nova, Qboa, Clorofina and Bright) and three 2.5% NaOCI-based products (Asfer, Iodontosul and UFPel), each one with five results. The group comprising 5.0% NaOCl solutions presents ten repetitions for tests conducted with five Iodontosul products and five products manufactured at Uso Indicado compounding pharmacy.

Kruskal-Wallis nonparametric test revealed statistically significant difference (p = 0.000) for at least one comparative analysis carried out between two pulp dissolution rate means of the groups mentioned. Dunnett's test revealed statistically significant differences between Table 2. Results of pulp dissolution rate (mg/s) for 0.5%, 1.0%, 2.5%, 2.0 to 2.5% and 5.0% NaOCI-based products that have completed the dissolution process.

		0.5% sodium	hypochlorite (Dak	in's solution)						
0.0048										
			0.0067							
0.0058										
			0.0089							
			0.0083							
1.0% sodium hypochlorite (Milton's solution)										
	Asfei			lodontosul						
	0.006	5	0.0072							
	0.010	7	0.0075							
	0.005	1	0.0056							
	0.006	7	0.0057							
0.0068				0.0068						
		2.5% and 2.0	to 2.5% NaOCI-ba	sed products						
NaOCI (2.5%)				NaOCI (2.0 to 2.5%) - hypochlorite-based bleaches						
Asfer (chlorinated soda)	lodontosul (Labarraque's solution)	UFPel (2.5% NaOCl)	Vida Nova	Qboa	Clorofina	Brilhante				
0.0238	0.0434	0.0101	0.0409	0.0147	0.0252	0.0156				
0.0254	0.0342	0.0126	0.0252	0.0073	0.0148	0.0261				
0.0215	0.0186	0.0101	0.0347	0.0131	0.0208	0.0183				
0.0174	0.0251	0.0059	0.0204	0.0136	0.0115	0.0296				
0.0202	0.0315	0.0090	0.0194	0.0138	0.0265	0.0231				
5.0% sodium hypochlorite										
	lodontosul (chlor	inated soda)	Uso Indicado							
0.0646				0.0653						
0.0759				0.0648						
0.0833				0.0613						
	0.055		0.0697							
0.0762				0.0687						

all comparative analysis carried out in pairs, except between 0.5% and 1.0% samples. Thus, complete bovine pulp dissolution estimates (20 mg in 15 milliliters) of 0.5, 1.0, 2.0 to 2.5 and 2.5 and 5.0% NaOCl solutions were 51, 50, 20 and 5 minutes, respectively.

Discussion

The method used in this study was based on the methodology proposed by Spanó⁸ and had already been used by Santos⁶ and Barbin.² It proved affordable, fast and efficient at evaluating bovine pulp tissue dissolving ability of different solutions. The experimental

model using bovine pulp has some advantages in comparison to the use of human teeth. For this reason, it has been adopted by several researchers.^{2,4,6,7,8,11}

Assessing the pulp dissolution rate of sodium hypochlorite-based products is enough to assess their ability to solve bovine pulp; however, estimating the time (in minutes) necessary to dissolve a 20-mg bovine pulp fragment in 15 ml of NaOCl solution allows one to assess a product's pulp dissolving ability according to common standards set by endodontic practice.

According to Haapasalo et al,¹² the ideal irrigating solution should have biocompatibility as one of its major characteristics, i.e., it should not irritate or damage periapical tissues. Although sodium hypochlorite fits most characteristics of an ideal irrigating solution, it has the disadvantage of toxicity. Due to being diluted, NaOCl solutions at lower concentrations carry lower risks of causing damage to periapical tissues in comparison to more concentrated solutions such as chlorinated soda, bleach and double-strength chlorinated soda.¹³ In contrast, the results yielded by the present research as well as by other studies found in the literature reveal that NaOCl at higher concentration is more effective in solving pulp tissue.

Leonardo¹⁴ found that an appropriate way to manage NaOCl toxicity and effectiveness would be to use the solution at 5.0% in the initial phase of crown-down preparation (cases of pulp necrosis and endodontic reintervention associated with chronic periapical periodontitis) while advancing into the cervical and middle thirds of the root canal. In the apical third, however, 2.5% NaOCl would be best used.¹⁴ NaOCl solutions at 0.5 and 1.0% present the lowest bovine pulp dissolving ability.

The aforementioned results are in agreement with those reported by Santos,⁶ but opposite to those reported by Spanó,⁸ Barbin,² Milano et al¹⁵ and Só et al¹⁶ who, by means of regression test and correlation between the concentration of sodium hypochlorite solutions and their ability to dissolve pulp tissue, established a direct, proportional relationship between concentration and pulp dissolution rate. In other words, the higher the concentration of the sodium hypochlorite solution, the greater its pulp dissolution rate.

NaOCl-based products at 0.5% and 1.0% reveal similar performances, which potentially suggests a significant influence of complementary components on the solution's bovine pulp tissue dissolving ability. Reducing pH levels by means of boric acid, used to produce Dakin's solution, may have improved 0.5% NaOCl dissolving ability, whereas stabilization with sodium chloride, used to produce Milton's solution, may have worsened 1.0% NaOCl dissolving ability.

Solutions at 2.5% and 2.0 to 2.5% showed intermediate dissolving ability statistically similar to each other. The presence of complementary components (e.g. stabilizers) was observed both in hypochloritebased bleaches and in 2.5% NaOCl-based products, whether industrialized or produced in compounding pharmacies. These data were obtained by reading the product label or exchanging information with the manufacturers. Importantly, potential influence of complementary components occurred in both cases, even though the FO-UFPel sample was produced by simple dilution.

Despite having a dissolution ability greater than 0.5 and 1.0% NaOCl-based products, 2.5% NaOCl-based products prepared at FO-UFPel were less satisfactory than other 2.5% NaOCl-based industrialized products. It is important to highlight that the production process of NaOCl-based products is considerably vulnerable, thereby pointing out to a great need to adhere to production protocols with emphasis on the acquisition of concentrated solutes and/or chemical reagents, as well as on titration and appropriate correction or dilution factors.

Some products do not have the ability to dissolve bovine pulp tissue, which suggests, particularly in cases of lower concentration products, instability of sodium hypochlorite. These cases require extra attention not only on aspects related to sodium hypochlorite transport and storage, but also to the potential adverse influence of complementary components, or the source of sodium hypochlorite, on the dissolution activity of NaOCI-based products.

Hypochlorite-based bleaches are registered by the Brazilian Health Surveillance Agency (ANVISA) as sanitizing products which are technically regulated by specific legislation.¹⁰ The dissolving ability of hypochlorite-based bleaches found in the present study should encourage manufacturers to register them as health-care products, which would authorize their use in Clinical Dentistry as an auxiliary solution used to treat root canals.

The use of an affordable and effective product in terms of dissolving ability and other properties^{1,17,18,19}, as it is the case of hypochlorite-based bleaches, would contribute to increase the health conditions of the general population, as government budget could be better employed in the Brazilian public healthcare system and endodontic treatment costs would be reduced in the private practice.

The 5.0% NaOCl-based products stood out among other products for having the greatest bovine pulp tissue dissolving ability. These findings are consistent with other authors.^{2,6,8} and corroborate Leonardo's⁹

discussion about the relationship established between toxicity and effectiveness of sodium hypochloritebased products.

Absence of bovine pulp tissue dissolution activity observed in 25% of the specimens tested may be related to some determining factors, namely: influence of complementary components (e.g. stabilizers); sodium hypochlorite source; the cation associated with hypochlorite anion (sodium or calcium); packaging; transport; storage; dilution and shelf life.

The findings of this study support the need for extreme care in acquiring NaOCl-based products, particularly with regard to various aspects such as strength; opaque and hermetic packaging; transport and storage; date of manufacture and expiration date; manufacturing method; evaluation of active chlorine (titration) and origin. All aforementioned aspects could be addressed by establishing effective communication with the manufacturers. Careless acquisition of NaOCl-based products without pulp tissue dissolution activity can compromise endodontic treatment predictability, prognosis and outcomes.

Conclusions

In the present study, 0.5 and 1.0% products proved to have the smallest bovine pulp tissue dissolving ability. Products at 2.5% and 2.0 to 2.5% had intermediate ability of dissolving bovine pulp while 5% sodium hypochlorite proved to have the greatest ability of dissolving pulp tissue among the products tested. The ability to dissolve bovine pulp tissue was not observed in all samples tested.

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