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Detection of Organochlorine Compounds Formed During the Contact of Sodium Hypochlorite with Dentin and Dental Pulp

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This study used gas chromatography-mass spectrometry (GC-MS) to detect the products formed during the contact of sodium hypochlorite (NaOCl) with bovine pulp and dentin. For analysis of the products formed in the volatile phase, 11 mg of bovine pulp tissue were placed in contact with 0.5%, 2.5% and 5.25% NaOCl until complete tissue dissolution occurred. The solid phase microextraction (SPME) fiber was exposed inside the container through the cover membrane and immediately injected into the GC-MS system. 30 mg of the of dentin were kept in contact with NaOCl, and then the SPME fiber was exposed inside the container through the cover membrane for adsorption of the products and injected into the GC-MS system. The same protocol was used for the aqueous phase. For analysis of the volatile compounds, the final solution was extracted using pure ethyl ether. The suspended particulate phase of the mixture was aspirated, and ether was separated from the aqueous phase of the solution. The ether containing the products that resulted from the chemical interaction of dentin and pulp with the NaOCl was filtered and then injected into the GC-MS system for analysis of the aqueous phase. The aqueous and volatile phases of both dentin and pulp showed the formation of chloroform, hexachloroethane, dichloromethylbenzene and benzaldehyde. In conclusion, organochlorine compounds are generated during the contact of dentin and pulp with NaOCl at concentrations of 0.5%, 2.5% and 5.25%.

Key Words: sodium hypochlorite, organochlorine, chloroform, hexachloroethane, dichloromethylbenzene.

Introduction

The irrigation of infected root canals is an important factor in the success of endodontic therapy. Sodium hypochlorite is a well-studied irrigant because of its antimicrobial effect, tissue dissolution capacity and acceptable biological compatibility at lower concentrations. Accidents with sodium hypochlorite may occur when the irrigant is in contact with periapical tissue or other soft tissues, which leads to severe inflammation (1-5).

The molecular formula of sodium hypochlorite is NaOCl, its molar mass, 74.44 g/mol, its density, 1.07-1.14 g/cm^3 and its boiling point, about 101°C. It is totally miscible in water, and its surface tension (about 70 dynes/cm) is high. Its concentration is directly proportional to its antimicrobial effect and tissue dissolution capacity and inversely proportional to its biological compatibility (3). In dilution tests, its minimal inhibitory concentration was lower than 1% for important microorganisms (*S. aureus*, *E. faecalis, P. aeruginosa and C. albicans*) (4). Its high pH explains its antimicrobial mechanism of action, similar to that of calcium hydroxide (5). It also affects the integrity of the cytoplasm membrane due to irreversible enzymatic inhibition, biosynthetic changes in cell metabolism and phospholipid destruction in lipid peroxidation. The formation of amino acid chloramine affects cell metabolism. Oxidation results in irreversible enzymatic inhibition of bacteria and replaces hydrogen with chlorine. Enzymes may be inactivated during the reaction of chlorine with amino groups and the irreversible oxidation of sulfhydryl groups of bacterial enzymes. The antimicrobial effect of NaOCl is explained by its action upon essential bacterial enzymes, which results in irreversible inactivation due to the action of hydroxyl ions and chloramines. Dissolution of organic tissues is observed during saponification, when NaOCl destroys fatty acids and lipids, which generates soap and glycerol (5).

Organochlorine compounds, usually found in small amounts in nature, originate from the contact of chlorine-based substances with organic tissue composed of carbon chains. They are neurotoxic, highly lipophilic, chemically stable and persistent in nature. Toxic to some plants and insects, they may be produced synthetically by the action of elemental chlorine upon petroleum hydrocarbons, and many have been widely used as pesticides. In the last decades, governmental agencies and environmental groups have made efforts to document contamination by organochlorine compounds and regulate their use, which has avoided dangerous concentrations, particularly in

human foods (6,7).

Because of their toxicity, studies have investigated their formation during water treatment (8-12) and their use as pesticides in agriculture (13,14). Although the environmental burden of some organochlorine pesticides has slowly decreased in several areas due to the restrictions to its use and production (15), the accumulation of these substances in the human organism still raises concerns (16). Some organochlorine-based pesticides, known as persistent organic pollutants, are hydrophobic, have long half-lives and tend to accumulate in the fatty tissue of animals and humans (17).

Chlorine (Cl2) is the most common disinfectant in water treatment because it is easy to use and has effective germicide properties and a low cost. However, chlorine also reacts with dissolved organic material and produces disinfection byproducts, such as trihalomethanes (chloroform) and haloacetic acids. Water disinfection, one of the main advances in public health, is responsible for decreases in mortality due to infectious diseases (18), but some disinfectant agents, such as chlorine, ozone, chlorine dioxide and chloramines, produce compounds that react with natural organic material during clean water production (19). Daily exposure to chlorinated water may be dangerous to human health because of the carcinogenic and mutagenic properties of these compounds. The consumption of chlorinated has been correlated with cancer risks (20). Baird (6) reported on the association between chlorinated water and cancer rates in several American communities. Public health problems caused by water disinfection are a major current concern in the entire world. Some studies demonstrated the health risks of organochlorine compounds for human beings, such as changes in cell metabolism and vital functions that lead to death by necrosis or in vivo apoptosis (8,9,19).

During root canal treatment, NaOCl is in direct and constant contact with dental pulp and dentin. As organochlorine compounds play in important role in human health, the toxic byproducts of the reaction of NaOCl and the organic matter inside the root canals should be investigated. This study used gas chromatography-mass spectrometry (GC-MS) to determine what compounds were produced as a result of the contact of different concentrations of NaOCl with bovine dental pulp and dentin.

Material and Methods

Organic Substrate Preparation

Freshly extracted bovine teeth were decoronated using a flexible mono-faced diamond disk (7010, KG Sorensen, São Paulo, SP, Brazil) and a N270 straight handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil). After that, the pulp tissue was removed from the roots using Hedströem files (Dentsply-Maillefer, Ballaigues, Switzerland), weighed (analytical scale, Mettler Toledo AG245, Canton, MA), separated into portions of about 11 mg each, stored in Eppendorf tubes (Quimis, Campinas, SP, Brazil) with deionized water (Quimis) and kept under refrigeration. The cement layer of the external surface of the root sections was removed using diamond tips (2068G, KG Sorensen), and the samples were then cut into small fragments and powdered using a ball mill (MLW, Germany) to obtain dentin microparticles. After that, bovine dentin was weighed (analytical scale, Mettler Toledo AG245), separated into portions of 30 mg and stored in Eppendorf tubes at room temperature.

Extraction Method for GC-MS

Dental pulp analysis: volatile extraction

Previously weighed pulp samples, separated and stored to preserve their features, were placed into capped glass bottles containing 2 mL of NaOCl at concentrations of 0.5%, 2.5% and 5.25%. The NaOCl samples were obtained from 10% NaOCl dilution in deionized water and immediately titrated using the iodometric method. The samples were divided into the following groups: (a) bovine pulp (11 mg) + 0.5% NaOCl (2 mL); (b) bovine pulp (11 mg) + 2.5% NaOCl (2 mL); (c) bovine pulp (11 mg) + 5.25% NaOCl (2 mL). All the samples were prepared in triplicate.

The bottles containing bovine pulp and the NaOCl solutions were kept under mixing (magnetic mixer without heating, Fisatom, São Paulo, Brazil) to ensure that the liquid was in contact with the entire external surface of the organic material until its total dissolution was confirmed by the presence of halogenated compounds. After that, still under mixing, SME fiber (65-µm PDMS/DVB SUPELCO, Bellefonte, PA, USA) attached to a holder was inserted into the recipient through a membrane in the cap, without touching the aqueous phase, and kept there for 15 min for the adsorption of the volatiles generated during the dissolution of organic material that was in touch with NaOCl at different concentrations.

After exposure (15 min), the fiber was removed from the bottle and immediately injected into the CG-MS system (QP 2010 plus; high injector AOC-20i Shimadzu, Tokyo, Japan) and kept there for 5 min for the analysis of volatile compounds.

Dentin analysis: volatile extraction

Dentin samples previously prepared, weighed and stored were placed in capped glass bottles with 2 mL of NaOCl at the concentrations described above and divided into the following groups: (a) bovine dentin (30 mg) + 0.5% NaOCl (2 mL); (b) bovine dentin (30 mg) + 2.5% NaOCl (2 mL); (c) bovine dentin (30 mg) + 5.25% NaOCl (2 mL). All the samples were prepared in triplicate.

The bottles containing bovine dentin and NaOCl solutions were kept under mixing (magnetic mixer without heating, Fisatom, São Paulo, SP, Brazil) for 15 min to ensure that the liquid was in contact with the entire external surface of the material. After that, still under mixing, SPME fiber (65-µm PDMS/DVB SUPELCO, Bellefonte, PA, USA) attached to a holder was inserted into the bottle using the membrane in the bottle cap, without touching the aqueous phase, and kept there for 15 min for the adsorption of the volatiles generated by dissolution of organic material in contact with NaOCl at different concentrations.

After exposure (15 min), the fiber was removed from the bottle, immediately injected into the CG-MS system and kept there for 5 min for the analysis of volatile compounds.

Dental Pulp and Dentin Analyses - Aqueous Phase Extraction

The same protocol was used to analyze the aqueous phase of pulp and dentin. After fiber removal for the analysis of volatile compounds, the final solution was extracted using pure ethyl ether at a standardized mixture of 2 mL of solution + 2 mL of ether (Merck Millipore, Darmstadt, Germany). The sample was kept under mixing (magnetic mixer without heating, Fisatom, São Paulo, Brazil) for 5 min to make sure the liquid was adequately mixed.

After mixing, the suspended particulate phase was aspirated using a micro-syringe (2-mL # 100² syringe, Hamilton CO. Gastight, Reno, NV) to separate ether from the aqueous phase of the solution. The ether, which contained the material resulting from the chemical interaction of the organic compounds of the pulp and dentin with the different NaOCl concentrations, was filtered (15-mm 0.45-µm organic regenerated cellulose filter, Ministart, Sartorius, Goettingen, Germany) and injected into the CG-MS system for analysis of the aqueous phase of the sample.

CG-MS Optimization to Detect Byproducts of Organic Material Decomposition by NaOCl

Different temperatures were used to ensure the best possible separation of decomposing products of organic material in NaOCl. Of the conditions assessed, the ideal chromatographic resolution was: initial oven temperature: 40 °C; injector temperature: 250°C; ramp: 40 °C for 2.5 min > 130 °C (at 35 °C/min) > 145 °C for 4 min.; flux control: linear speed; pressure: 49.7 kPa; total flux: 14 mL/min; split ratio: 10:1.

Sample Calibration Curve

The calibration curve was built using a standard method. Control 1 (SPME fiber): the fiber was injected for CG-MS analysis as soon as removed from casing. The results indicated that no substances that might affect results were detected in the SPME fiber; Control 2 (5.25% NaOCl): the fiber was placed into a closed environment containing 5.25% NaOCl, exposed for 15 min without contact with liquid, and injected into the CG-MS system for analysis. The results revealed that low oscillations were within the standard deviation, which showed that 5.25% NaOCl had no substances that might affect results; Control 3 (bovine pulp): the fiber was placed into a closed environment containing bovine pulp, kept there for 15 min without contact with the sample, and then injected into the CG-MS system. The results indicated that the bovine pulp had no substances that might affect results; Control 4 (bovine dentin): the fiber was placed into a closed environment containing bovine dentin, kept there for 15 min without contact with the sample, and then injected into the CG-MS system. There were no substances in bovine dentin that might affect results; Control 5 (ethyl ether): the fiber was placed into a closed environment containing ethyl ether, kept there for 15 min without contact with the sample and then injected into the CG-MS system. The peak retention time of 1.6 min indicated the presence of volatized ethyl ether adsorbed by fiber; no other substances or products that might affect results were found.

Results

The GC-MS results of compounds formed during the contact of NaOCl with organic material (pulp and dentin) will be described in three sections: 1) volatile extraction; 2) aqueous phase extraction; 3) analysis of GC peaks of volatile compounds (NaOCl concentration and products generated).

Volatile Extraction

Dentin

The GC-MS results of volatile extraction of compounds formed during the contact of NaOCl at different concentrations with bovine dentin are shown in Figure 1A. Peak retention time (RT) was 2.15 min, which corresponds to chloroform, and mass load (m/z) was 83.47, which corresponds to an organochlorine (Fig. 1B). The second peak RT, 5.32 min, corresponds to benzaldehyde (m/z = 106.77.51) (Fig. 1C). After that, there was a third peak at RT = 6.30 min, which indicated the detection of another organochlorine, hexachloroethane (m/z = 201.166.117.94) (Fig. 1D). A last peak retained at RT = 6.7 min corresponded to another organochlorine formed during the contact of NaOCl with the organic material of bovine dentin: dichloromethylbenzene (m/z = 125.89.63) (Fig. 1E).

Pulp

The GC-MS results of volatile extraction of compounds formed during the contact of NaOCl at different concentrations with bovine pulp are shown in Figure 2. The compounds formed during the contact of NaOCl with bovine

pulp and dentin were the same: chloroform, benzaldehyde, hexachloroethane and dichloromethylbenzene.

Aqueous Phase Extraction

The GC-MS results of the extraction of the aqueous phase of compounds formed during the contact of NaOCl at different concentrations with bovine dentin and pulp are shown in Figure 3. A peak between 1-2min corresponded to ethyl ether. The black line corresponds to the control graph of the solvent used, which confirmed its identification

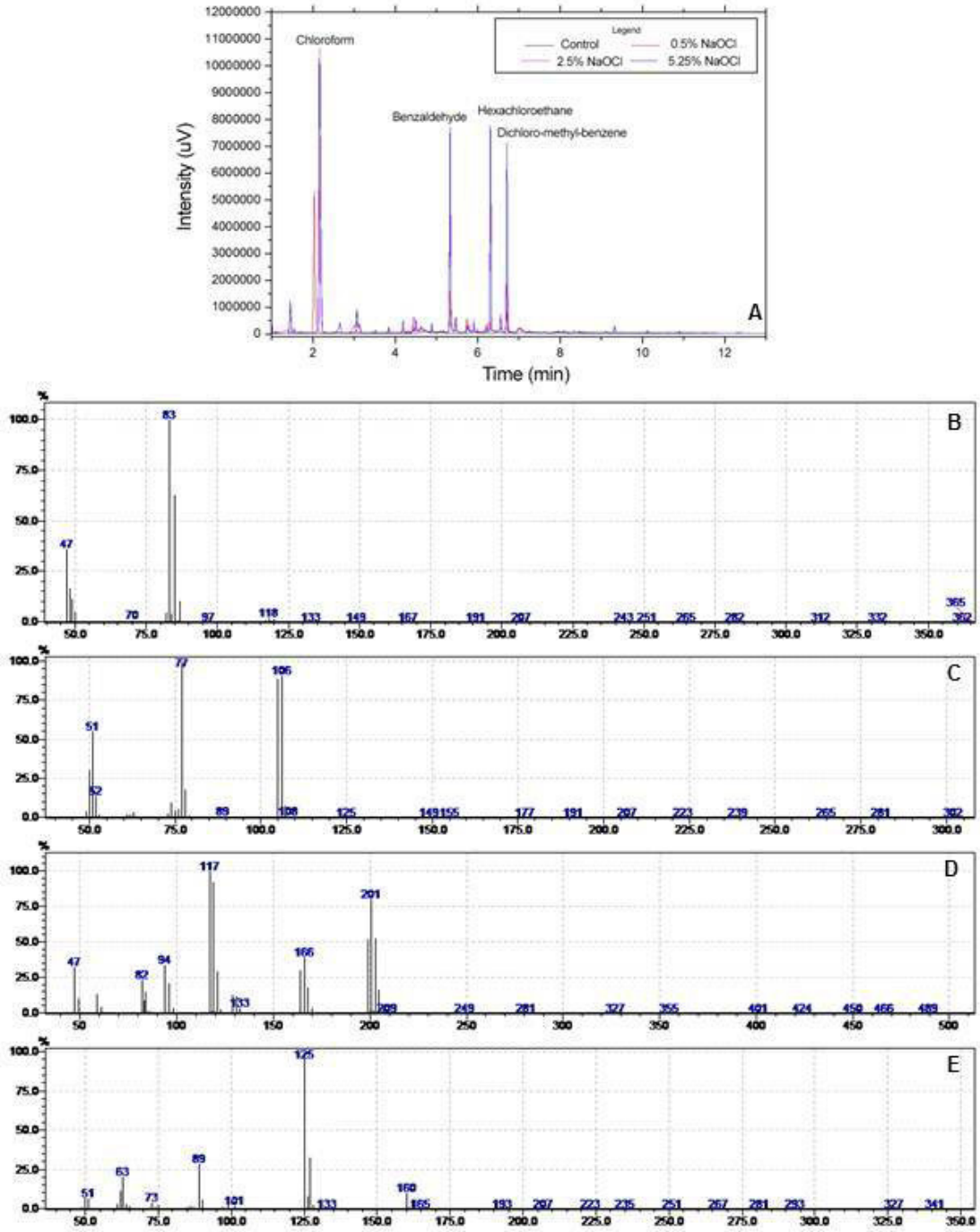

Figure 1. A: Initial chromatographic analysis of originated products in reactions of NaOCl and bovine dentin. B: Mass spectrum of peak TR = 2.15 min from chloroform, obtained after fiber desorption. C: Mass spectrum of peak TR = 5.32 min from benzaldehyde, obtained after fiber desorption. D: Mass spectrum of peak TR = 6.3min from hexachloroethane, obtained after fiber desorption. E: Mass spectrum of peak TR = 6.7min from dichloromethylbenzene, obtained after fiber desorption.

by CG-MS. After identification, GC-MS detected no other response to ethyl ether.

The results of the analysis of volatile compounds in both bovine dentin and pulp samples revealed that, in aqueous phase, the same compounds were formed in the different substrates: chloroform, benzaldehyde, hexachloroethane and dichloromethylbenzene at RT of 2.15 min, 5.32 min, 6.3 min and 6.70 min.

Analysis of GC Peaks of Volatile Compounds - NaOCl Concentration and Compounds Formed

Dentin

Table 1 shows the areas of each peak of Figure 1A, which correspond to the compounds formed during the contact of bovine dentin with NaOCl at different concentrations. The height of peaks in Figure 1A does not correspond to the amount of each compound formed. The values in the y-axis of Figure 1A are the intensity (uV) of each compound detected using CG-MS. Thus, the amount formed of each compound is expressed by peak area. At RT = 2.15 min in Figure 1A, which corresponds to chloroform, the highest peak was found for 2.5% NaOCl, followed by that for 5.25% NaOCl, and the lowest, for 0.5% NaOCl, but the amount of compound formed was proportional to NaOCl concentration. In other words, the lowest NaOCl concentration (0.5%) had a smaller area (10681137) of chloroform formed during the reaction, followed by the area of 24501360, which corresponded to chloroform formed during contact of 2.5% NaOCl with bovine dentin, whereas the largest area was 24856101, which corresponded to the organochlorine formed for 5.25% NaOCl, as shown in Table 1. Therefore, the formation of chloroform was directly proportional to NaOCl concentration.

The analysis of benzaldehyde revealed that at RT = 5.32 min (Fig. 1A) the peaks generated according to the variation of each NaOCl concentration were proportional to peak area. The lowest peak (0.5% NaOCl) also had the smallest area (2045827), followed by the area of the 2.5% NaOCl peak (8417137), whereas the highest peak (12298806) corresponded to 5.25% NaOCl. The peaks of benzaldehyde, hexachloroethane and dichloromethylbenzene were also proportional to NaOCl areas and concentrations. Hexachloroethane (RT = 6.30 min) had the lowest peak when 0.5% NaOCl was used, and its area was 511172. NaOCl at 2.5%, the second highest concentration, had the second highest peak and the second largest area (8887362). The solution with the highest concentration, 5.25%, had the highest peak in Figure 1 and an area of 12298806. Dichloromethylbenzene detected at RT = 6.7 min had the lowest peak when NaOCl concentration was 0.5%, and its area was 1208900; when 2.5% NaOCl was used, the area was 3045170; and the largest area (11691997) was found for 5.25% NaOCl.

Pulp

Table 2 shows the areas of the peaks in Figure 2, which correspond to the compounds formed during the contact with NaOCl at different concentrations. In the first peak of Figure 2 (RT = 2.15 min), which corresponds to chloroform, the highest peak is the GC-MS result for 2.5% NaOCl, followed by the peak for organochlorine formation when in contact with 0.5% NaOCl, whereas the lowest peak corresponded to bovine dentin in contact with 5% NaOCl; however, the amount of chloroform does not correspond to the peaks, as it increases with concentration. The smallest area (7442200) corresponded to the second highest peak (0.5% NaOCl), followed by the second largest area (12190135), seen in Table 2, and whose peak, the highest, is shown in Figure 2, whereas the largest area (12382353) in Table 2 corresponds to the lowest peak. The peaks in Figure 2 that correspond to benzaldehyde are seen at RT = 5.32 min and confirm that the higher the peak, the larger the area in Table 2 and the higher the concentration of NaOCl. The area of the lowest peak, which corresponded

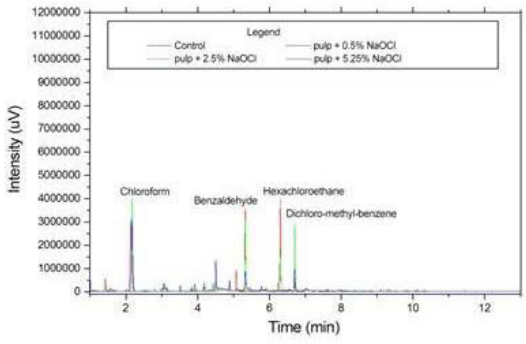

Figure 2. Immediate analysis of volatile products originated in reactions of NaOCl and bovine pulp.

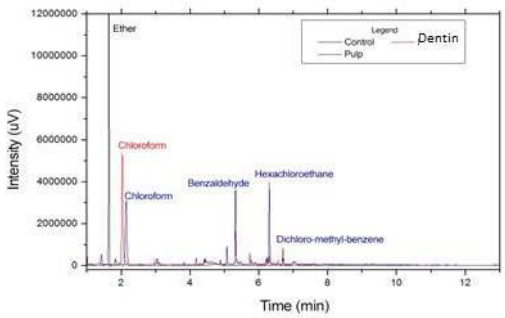

Figure 3. Extraction of ethyl ether of aqueous phase of originated products in reaction between 5.25% NaOCl / bovine dentin and 5.25% NaOCl + bovine pulp.

to 0.5% NaOCl, was 1837003, followed by that of 2.5% NaOCl (area = 4872823); and the highest peak had an area of 6867971 and corresponded to benzaldehyde formed during the contact with 5.25% NaOCl.

The GC-MS peaks of hexachloroethane at RT = 6.30 min also vary according to area and NaOCl concentration. The smallest area (1393254) and lowest peak corresponded to the lowest concentration of NaOCl in this study (0.5% NaOCl). The second highest peak corresponded to 2.5% NaOCl (2791955), and the largest area (8957649) was found for the highest peak when the 5.25% concentration was used.

At RT = 6.70 min (Fig. 2), which corresponded to peaks of dichloromethylbenzene, the amount of organochlorine did not increase with NaOCl concentration, differently from all the other peaks in the results. The highest peak with the largest area (4943370) was formed when the sample was in contact with 2.5% NaOCl. When using 0.5% NaOCl, the lowest concentration in this study, a second highest peak and second largest area were found for dichloromethylbenzene (1574741). The use of 5.25% NaOCl corresponded to the lowest peak and smallest area (1115776), as shown in Figure 2.

Discussion

When treating infected root canals, cleaning and shaping, together with the action of NaOCl, reduce the remaining microbiota and ensure better prognoses. NaOCl has been studied and indicated at different concentrations (1-5).

The results of this study showed that the contact of NaOCl with dentin and pulp results in the formation of organochlorine compounds. Regardless of NaOCl concentration (0.5%, 2.5% or 5.25%), the same compounds were formed, and concentration was directly proportional to amount of each compound. Analysis of the aqueous and volatile phases of both dentin and pulp revealed the formation of chloroform, hexachloroethane, dichloromethylbenzene and benzaldehyde.

GC-MS was effective in detecting products formed during the contact of NaOCl with bovine dentin and pulp. Chromatography is a physical separation method in which compounds are distributed into two phases, one stationary and one that moves in a defined way. The mixture with the compounds to be separated is dissolved during the mobile phase. As the mobile phase passes through the stationary phase, some compounds are substantially retained, and, therefore, move slowly. Meanwhile, other compounds interact weakly with the stationary phase and are transported more easily by the mobile phase. These differences in mobility are used to separate compound mixtures and analyze them qualitatively or quantitatively, using, for example, spectrophotometry or mass spectrometry (10). In a GC-MS system, samples are bombarded by electrons and broken, generating positive and negative ions and radicals. The differences in the

Table 1. Areas of each peak of Figure 1A, corresponding to the compounds formed during the contact of bovine dentin with NaOCl at different concentrations

Peak	NaOCl (%)	Retention time (min)	Area
	0.5		10681137
Chloroform	2.5	2.15 (peak 1)	24501360
	5.25		24856101
	0.5		2045827
Benzaldehyde	2.5	5.32 (peak 2)	8417137
	5.25		11931805
	0.5		511172
Hexachloroethane	2.5	6.30 (peak 3)	8887362
	5.25		12298806
	0.5		1208900
Dichloro-methyl-benzene	2.5	6.70 (peak 4)	3045170
	5.25		11691997

Table 2. Areas of the peaks in Figure 2, which correspond to the compounds formed during the contact with NaOCl at different concentrations

Peak	NaOCl (%)	Retention time (min)	Area
	0.5		7442200
Chloroform	2.5	2.15 (peak 1)	12190135
	5.25		12382353
	0.5		1837003
Benzaldehyde	2.5	5.32 (peak 2)	4873283
	5.25		6867971
	0.5		32675
Hexachloroethane	2.5	6.30 (peak 3)	2791955
	5.25		8957649
	0.5		1574741
Dichloro-methyl-benzene	2.5	6.70 (peak 4)	4943370
	5.25		1115776

relation of mass/load of ions generated separate them (21,22).

This study identified organochlorine compounds (chloroform, hexachloroethane and dichloromethylbenzene) formed during the contact of NaOCl with bovine pulp and dentin. Chloroform, or trichloromethane, an organochlorine unduly called formaldehyde trichloride, is highly refractive, nonflammable and volatile and has a high molecular weight, characteristic smell and, when in its liquid state, a sweet taste. It solidifies at -63.5 °C and hits boiling point at 59 °C (6-10). This study found a greater amount of chloroform in the analysis of the volatile phase than of the aqueous phase. This is explained by the high volatility of this organochlorine. Smyth et al. (23) studied the effect of chloroform on rats and found that it is toxic to living organism, and that the lethal oral dose for that species was 2.18 g/kg. When inhaled in high doses, it may cause hypotension, respiratory and cardiac depression and even death. Although this substance is carcinogenic, it is still used in fats, oils, rubber, waxes, gutta-percha solvent, cleaning agents and fire extinguishers, where it reduces the freezing temperature of carbon tetrachloride. Hexachloroethane, an organochlorine that smells like camphor, is soluble in chloroform, alcohol, benzene, ether and oils, and is insoluble in water. Found in its crystal form, it sublimates without melting (6-10). The IV lethal dose in dogs is 325 mg/kg (24). In humans, it may cause moderate irritation to skin and mucous membranes. Dichloromethylbenzene is an organochlorine that should be further evaluated, although benzenes are toxic substances. Benzaldehyde, another substance formed during this study, is not an organochlorine and is found in grain kernels. In its liquid state, it is yellow, has a high refraction value and a characteristic smell and must be kept in a closed recipient and protected from light. Its boiling point is about 25° C, and its freezing point, 56.5 °C. The lethal oral dose in rats is 1300/1000 mg/kg (25). It is used in the production of dyes and perfume and as solvents and aromatic agents. At high concentrations, it may cause contact dermatitis (6-10).

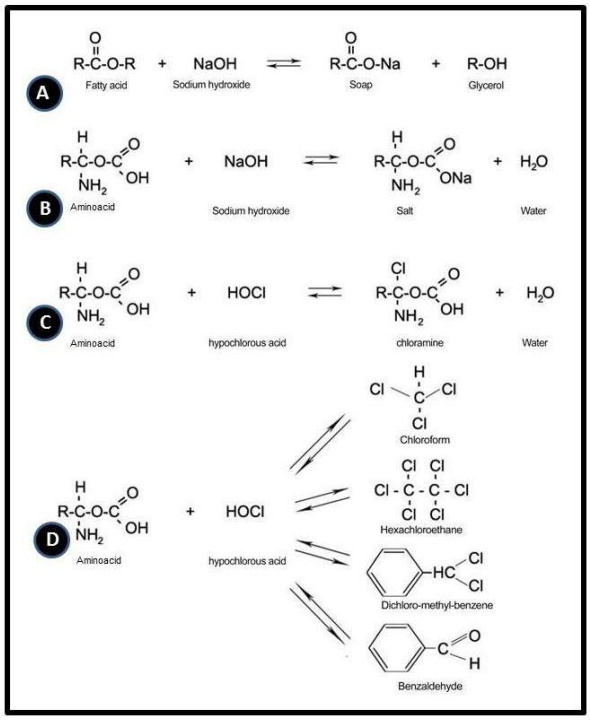

Figure 4. Chemical reactions between pulp tissue and NaOCl. A: saponification reaction. B. Neutralization reaction. C: Chloramination reaction. D: Byproducts generated: chloroform, benzaldehyde, hexachloroethane and dichloromethylbenzene).

Sodium hypochlorite has a dynamic balance, as shown in NaOCl + H_2O « NaOH + HOCl « Na+ + OH- + H+ + OCl (3,5). The analysis of some chemical reactions of organic tissues and NaOCl conducted so far are shown in Figure 4A-C (5). The interpretation of these chemical reactions demonstrates that NaOCl acts as an organic and fat solvent that degrades fatty acids and transforms them into fatty acid salts (soap) and glycerol (alcohol), which reduces the surface tension of the remaining solution (saponification, Fig. 4A). NaOCl neutralizes amino acids and forms water and salt (neutralization, Fig. 4B). As hydroxyl ions are lost, its pH is lowered. Hypochlorous acid, a substance found in NaOCl solutions, acts as a solvent when in contact with organic tissues and releases chlorine, which, when combined with the protein amino group, forms chloramines (chloramine formation, Figure 4C). Hypochlorous acid (HOCl-) and hypochlorite ions (OCl-) lead to amino acid degradation and hydrolysis (5). This study found that, in the volatile and the aqueous phases, the contact of NaOCl with bovine pulp and dentin led to the formation of four byproducts: chloroform, benzaldehyde, hexachloroethane and dichloromethylbenzene. Three of these products have chemical structures that characterize organochlorine compounds: chloroform, hexachloroethane and dichloromethylbenzene (Fig. 4D).

NaOCl has been one of the most common irrigant agents in root canal treatment for more than a century. The positive aspects of its use are associated with its organic and necrotic tissue dissolution properties, whitening action, antimicrobial potential, saponification, transformation of amines into chloramines and deodorization. On the other hand, the complete disruption and elimination of the bacterial biofilm remains a challenge for new studies, as well as the fact that no operator is immune to accidents that may occur during the use of this substance not in the root canal (1-5).

Therefore, its use in dental clinical procedures should be reviewed frequently because both the dentist and the patient may accidentally inhale the volatile phase

of byproducts formed during the contact of NaOCl with organic substrates and be exposed to organochlorine compounds that represent a threat to human health, as they tend to accumulate in human adipose tissue (8-10). Further studies should be conducted to analyze the potential tissue damage caused by organochlorine compounds formed during the contact of NaOCl with organic substrates. One of aspects that should be investigated is the effect of the amount of organochlorine compounds produced during the irrigation and manipulation of NaOCl, which can be associated with actual health risks for dentists and patients.

This study showed that organochlorine compounds are formed during the contact of NaOCl with organic substrates (pulp or dentin). The generation of these byproducts occurred at all concentrations (0.5%, 2.5% and 5.25%). The amounts of all byproducts were directly associated with NaOCl concentrations, except for dichloromethylbenzene in the volatile phase of the test for bovine pulp.

Resumo

Este estudo utilizou a cromatografia gasosa acoplada a espectrometria de massa (CG-MS) para detectar os produtos que se formaram durante o contato de hipoclorito de sódio (NaOCl) com polpa dental bovina e dentina. Para a análise dos produtos formados na fase volátil, 11 mg de polpa bovina foram colocados em contato com 0,5 % , 2,5 % e 5,25 % de NaOCl, até à dissolução completa dos tecidos. A fibra de microextração em fase sólida (SPME) era exposta dentro do recipiente através da membrana da tampa, por 15 minutos, para a adsorção dos produtos formados e imediatamente injetada no CG-MS para análise. Para a análise da dentina, 30 mg do de amostras foram mantidas em contacto com o NaOCl, por 15 min, e então a fibra de SPME era exposta no interior do recipiente através da membrana de cobertura para a adsorção dos produtos e injectado no sistema de GC-MS. O mesmo protocolo foi utilizado para a fase aquosa. Para a análise dos compostos voláteis, a solução final foi extraída com éter etílico puro. A fase de partículas em suspensão da mistura foi aspirada, e o éter foi separado da fase aquosa da solução. O éter contendo os produtos que resultaram da interacção química da dentina e polpa com hipoclorito de sódio foi filtrado e, em seguida, injectado no sistema GC-MS para análise da fase aquosa. As fases aquosas e voláteis de dentina e polpa mostraram a formação de clorofórmio, hexacloroetano, dichloromethylbenzene e benzaldeído. Compostos organoclorados são gerados durante o contacto da dentina e polpa com hipoclorito de sódio em concentrações de 0,5 % , 2,5 % e 5,25 %.

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