

INFLUENCE OF IODOFORM ON ANTIMICROBIAL POTENTIAL OF CALCIUM HYDROXIDE

INFLUÊNCIA DO IODOFÓRMIO NO POTENCIAL ANTIMICROBIANO DO HIDRÓXIDO DE CÁLCIO

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ABSTRACT

The purpose of this research was to verify the influence of Iodoform on antimicrobial potential of calcium hydroxide. *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis*, *C. albicans* were the biological indicators. The substances tested were: calcium hydroxide + saline; calcium hydroxide + Iodoform + saline; Iodoform + saline. For the agar diffusion test, 18 Petri plates with 20 ml of BHI agar were inoculated with the microbial suspensions. Fifty-four cavities were made and filled with the substances tested. The diameters of microbial inhibition were then measured. In direct exposure test, 162 #50 sterile absorbent paper points were immersed in the experimental suspensions for 5 min, and covered with the pastes. At intervals of 24, 48 and 72 hours, the paper points were immersed in 10 ml of Letheen Broth, followed by incubation at 37°C for 48h. Microbial growth was evaluated by turbidity of the culture medium. A 0.1 ml inoculum obtained from the Letheen Broth was transferred to 7 ml of BHI, and incubated at 37°C for 48h. Bacterial growth was again evaluated by turbidity of the culture medium. The calcium hydroxide associated with the saline or the iodoform plus saline showed antimicrobial effectiveness in both experimental methods. The iodoform paste presented antimicrobial ineffectiveness for the agar diffusion test on all biological microorganisms and for the direct exposure test on *B. subtilis* and on the mixture.

Uniterms: Calcium hydroxide; Iodoform; Intracanal medicaments.

RESUMO

O objetivo deste estudo foi verificar a influência do Iodofórmio no potencial antimicrobiano do hidróxido de cálcio. *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis*, *C. albicans* foram os indicadores biológicos. As substâncias testadas foram: hidróxido de cálcio + solução salina; hidróxido de cálcio + iodofórmio + solução salina; iodofórmio + solução salina. Para o teste de difusão em ágar, 18 placas de Petri contendo 20 ml de ágar BHI foram inoculadas com as suspensões microbianas. Cinquenta e quatro cavidades foram feitas e preenchidas com as substâncias testadas. Os diâmetros da inibição microbiana foram então mensurados. No teste de exposição direta, 162 pontas de papel absorvente número 50 esterilizadas foram imersas nas suspensões experimentais por 5 minutos, e cobertas pelas pastas testadas. Em intervalos de 24, 48 e 72 horas, as pontas de papel foram imersas em 10 ml de Letheen Broth, seguido de incubação a 37°C por 48 horas. O crescimento microbiano foi avaliado pela turvação do meio de cultura. Um inóculo de 0.1 ml obtido do Letheen Broth foi transferido para 7 ml de BHI, e incubado a 37°C por 48h. o crescimento bacteriano foi novamente avaliado pela turvação do meio de cultura. As pastas contendo hidróxido de cálcio e solução salina, hidróxido de cálcio-iodofórmio e solução salina mostraram significativa atividade antimicrobiana nos métodos experimentais estudados. A pasta contendo iodofórmio e solução salina foi inefetiva pelo teste de difusão em ágar e, também, por exposição direta, para o *B. subtilis* e a mistura.

Unitermos: Hidróxido de cálcio; Iodofórmio; Medicação intracanal.

INTRODUCTION

Chemical and biologic dynamics of any intracanal medication on tissue and bacteria have promoted significant discussion about this theme. An important factor to consider before choosing any intracanal medication is the knowledge of its mechanism of action and of the predominant microorganisms in endodontic infections.

It is interesting to know that any microorganism infecting the root canal has potential to initiate apical periodontitis. Although the individual species in the endodontic microbiota are usually of low virulence, collectively they are pathogenic due to a combination of factors. These factors include interactions with other microorganisms in the root canal, when they develop synergistically beneficial partners; release endotoxins; produced enzymes that damage host tissues; as well as interfere and evade host defenses²².

It is necessary to consider that the success of endodontic treatment is directly influenced by elimination of the microorganisms in infected root canals.

However, different intracanal medicaments have been proposed¹⁻²⁵. Calcium hydroxide has been shown by longitudinal scientific evidence to be the best therapeutic option as intracanal dressing^{6-8,10-12,16}.

The basic principle action of calcium hydroxide involves the ionic dissociation into hydroxyl ions and calcium ions and its positive effect on microorganisms and tissue healing process.

Estrela, et al.¹² (1995) studying the mechanism of action of hydroxyl ions on microbial enzymes in the cytoplasmic membrane reported that promotion changes in the transport of nutrients and in the structure of organic components can be responsible for its destruction. This interference in biosynthetic processes that are essential to microorganisms' lives can also be analyzed through the process of lipid peroxidation and the influence of hydroxyl ions. Thus, the quantity of existing hydroxyl ions can lead to enzymatic deactivation of bacteria. The mechanism of action of calcium hydroxide's pH in the control of bacterial enzymatic activity allowed Estrela, et al.⁸ (1995) to suggest the hypothesis of an irreversible bacterial enzymatic inactivation under extreme conditions of pH for a long period of time and also a temporary bacterial enzymatic inactivation with the restoration of normal activity when the pH returns to the ideal level for enzymatic activity.

Several works^{2,7,17,21,23} have studied the mixture of other substances to calcium hydroxide with the purpose of improving some of its properties. Among these additional substances are vehicles that can speed up or slow down ionic dissociation, substances that aid the filling of pulpal cavity by means of their consistency, substances used as antimicrobial medium and media that enhance radiopacity (distilled water, saline solution, propylene glycol, camphorated paramonochlorophenol, chlorhexidine, glycerin, corticosteroid-antibiotic, antibiotics, anesthetic solution, methylcellulose, glycerin, detergent, iodoform, barium sulfate).

Iodoform (triiodomethane CHI_3 ; molecular weight 393.78; atomic weight 126,9044) is composed of some powder with bright hexagonal crystals of lemon yellow color, with penetrating and persistent smell, little soluble in water (1:10.000), soluble in alcohol (1:60), and soluble in ether (1:75). It decomposes releasing iodine in nascent state (96,7% of iodine). Iodoform is soluble in fatty acids, little stable and it decomposes easily facing organic substances in decomposition²³.

Compounds that contain iodine are very employed for infection control in Dentistry. Iodine action gives them a high reactivity by precipitating proteins and oxidizing essential enzymes. In the presence of organic and inorganic substances the activity of iodine may be reduced. Iodine can be dissolved in aqueous potassium iodide, alcohol or make an assembly with a transporter (known as iodoform). Iodoforms are iodine compounds (prepared with low surface tension substance and stabilizer). They are classified as disinfectants of intermediate level (these compounds are also used as antiseptics). An iodine product that is very used as an antiseptic pre-surgical solution is PVPI (10% polyvinylpyrrolidone iodine in alcoholic solution with 1% of active iodine)⁶.

All over the years, pastes containing iodoform were exhaustively indicated as antiseptics due to iodine release in nascent state when in contact with secretions or endodontic infections, as shown by several works^{4,18,19,23}. In other studies^{2,3,14,15,20,21,25}, for different reasons, iodoform has been used mixed to calcium hydroxide paste. The purpose of this research was to verify the influence of iodoform on antimicrobial potential of calcium hydroxide against microorganisms with different structural characteristics (Gram-positive coccus, Gram-negative rods, Gram-positive rods, and a Yeast) by using two experimental methods (agar diffusion test and direct exposure test).

MATERIALS AND METHODS

Test organisms

Five biological indicators obtained from the American Type Culture Collection were used for this experiment: *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), one yeast, *Candida albicans* (ATCC 10231) and a mixture of these.

The strains were inoculated in 7 ml of Brain Heart Infusion (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 24h. The 5 biological indicators were cultivated on the surface of Brain Heart Infusion Agar (BHIA; Difco Laboratories, Detroit, MI, USA) at the same incubation conditions; microbial cells were re-suspended in saline to give a final concentration of about 3×10^8 cells/ml, similar to that of tube #1 of the MacFarland scale. One ml of each of these pure suspensions was used to obtain a mixture of the test microorganisms.

Substances tested

Three substances were tested in paste form, corresponding to the consistency of toothpaste: calcium hydroxide (P.A., Quimis, Mallinkrodt Inc., St. Louis, MO, USA) + saline (CHS); calcium hydroxide + iodoform (Biodinâmica, Ibiporá, PR, Brasil) (1:1) + saline (CHIS); iodoform + saline (IS).

Agar diffusion test

In this test, 18 Petri plates with 20 ml of BHIA were inoculated with 0.1 ml of the microbial suspensions, using sterile swabs that were spread on the medium, obtaining growth in junction. Three cavities (4mm in depth and 4mm in diameter) were made in each agar plate (total=54), using a copper coil and completely filled with the substances tested. The plates were maintained for 1h at room temperature, and then incubated at 37°C for 48h. The diameters of microbial inhibition were then measured around the cavities containing the substances. Positive and negative controls were done, maintaining the plates inoculated and without inoculum for the same periods and under identical incubation conditions. All assays were carried out under aseptic conditions.

Direct exposure test

For this test, one hundred and sixty two # 50 sterile absorbent paper points (Tanari, Tanariman Indústria, Ltda., Manacaru, AM, Brazil) were immersed in the experimental suspensions for 5 min, and were then placed on Petri plates and covered with the pastes. At intervals of 24, 48 and 72 hours, 18 absorbent paper points were removed from contact with the pastes, individually transported, and immersed in 10 ml of Lethen Broth (LB, Difco Laboratories, Detroit, MI, USA), and subsequently incubated at 37°C for 48h. Microbial growth was analyzed by turbidity of the culture medium. Subsequently an inoculum of 0.1 ml obtained from Lethen Broth was transferred to 10 ml of BHI, under identical incubation conditions. Microbial growth was also evaluated by turbidity of the culture medium. Gram stain of BHI cultures was used to verify the contamination and growth was

determined with macroscopic and microscopic (Gram stain) examination. All assays were carried out in triplicate under aseptic conditions.

RESULTS

The results obtained are shown at Tables 1 and 2. The calcium hydroxide associated with the saline or the iodoform plus saline, showed antimicrobial effectiveness in both experimental methods. Analysis of the numerical data showed no significant differences between both pastes containing calcium hydroxide, and significant differences with iodoform paste (Kruskal-Wallis test; $H=39.45$, $p=0.127$). The iodoform paste presented antimicrobial ineffectiveness for the agar diffusion test on all biological microorganisms and for the direct exposure test on *B. subtilis* (aerobic Gram-positive rods, spore-forming) and on the association.

DISCUSSION

Despite of the obtained results, it is wise to be careful about the possibility of direct extrapolation of the in vitro results to clinical activity. A detailed analysis of what is possible to obtain with this study method is necessary and convenient.

Aydos and Milano¹ (1984) questioned the antiseptic ability of iodoform for its use in root canal. They concluded that iodoform provides radiopacity to calcium hydroxide pastes and that it has no antibacterial action in vitro, presenting great divergence concerning its action in vivo. Iodoform's ability of biological stimulation, due to lack of investigations, may only be considered an hypothesis. Bramante, et al.² (1986) analyzed perforations in premolars of adult dogs, filled with calcium hydroxide paste and iodoform with different vehicles (physiologic solution, polyethylene glycol 400 and lipiodol). The perforations filled with calcium hydroxide paste plus iodoform and polyethylene glycol presented the best results in the histological examination with significant minor inflammatory process and the best reparative evolution. Siqueira, et al.²⁴ (1997), evaluating the antibacterial activity of calcium

TABLE 1- Means of the diameters (in mm) of the inhibition zones of the pastes by the agar diffusion test

Microorganisms Pastes	<i>S. aureus</i> AGPC	<i>E. faecalis</i> AGPC	<i>P. aeruginosa</i> AGNR	<i>B. subtilis</i> AGPR ^x	<i>C. albicans</i> Yeast	Mixture
CHS**	7	9	5.5	8	10	5
CHIS**	9	9	8	8	8.5	8
IS*	0	0	0	0	0	0

(CHS – Calcium Hydroxide + Saline; CHIS - Calcium Hydroxide + Iodoform + Saline; IS - Iodoform + Saline)
(AGPC = aerobic Gram-positive coccus; AGNR = aerobic Gram-negative rods; AGPR^x = aerobic Gram-positive rods, spore-forming)

(Kruskal-Wallis test; $H=39.45$, $p=0.127$, no statistical differences**, statistical difference*).

hydroxide/ camphorated paramonochlorophenol/ glycerin paste containing different amounts of iodoform on obligate anaerobic bacteria, concluded that the addition of iodoform to this paste did not influence their bacterial properties. Daniel, et al.⁵ (1999) in a literature review on employment of iodoform in endodontics did not find any laboratorial or clinical study that justifies the employment or abandonment of iodoform in the treatment of refractory periapical lesions.

Based on Estrela and Holland's⁹ (2003) discussion of calcium hydroxide properties, supported by scientific evidence, it is possible to state that: 1. Dentin is considered the best pulpal protector, and calcium hydroxide has proved, through numerous studies, its capability of inducing the formation of a mineralized bridge over pulpal tissue. 2. It is necessary, whenever possible, to allow time for calcium hydroxide paste to manifest its potential of action on the microorganisms present in endodontic infections. The maintenance of a high concentration of hydroxyl ions can change bacteria enzymatic activity and promote its inactivation. 3. The site of action of hydroxyl ions of calcium hydroxide includes the enzymes in the cytoplasmic membrane. This medication has a large scope of action, and therefore is effective on a wide range of microorganisms, regardless of their metabolic capability. In microbiology, cytoplasmic membranes are similar, independent from microorganisms morphological, tinctorial and respiratory characteristics, which means that this medication has a similar effect on aerobic, anaerobic, Gram-positive and Gram-negative bacteria. 4. Calcium hydroxide as a temporary dressing when used between appointments promotes better results on the periapical healing process than treatment in a single appointment.

In the present study, the data obtained by experimental

methods (agar diffusion test and direct exposure test) demonstrated that iodoform did not increase the antimicrobial effect of calcium hydroxide plus saline solution.

Iodoform presents excellent radiopacity, what leads some professionals to mix it with other intracanal medications.

It may be noted that the correct filling of root canal is as important as the effectiveness of calcium hydroxide, since the lack of direct contact of this dressing interferes with its mechanism of action^{8,10,12}. However, when the root canal is well filled, dentin and calcium hydroxide paste in association with saline solution clinically present themselves with the same radiopacity, causing the disappearance of root canal light¹⁰.

The literature reports that iodoform's action occurs from releasing iodine, which gives it high reactivity by precipitating proteins and oxidizing essential enzymes. It has great employment in infection control in Dentistry and is also considered a disinfectant of intermediate level. Even so, further studies are necessary to clarify the real mechanism of action of this medication in endodontic infections and periapical tissues. Time of effectiveness, diffusion in dentinal tubules, ability of neutralizing toxins (hydrolyzed LPS) and stimulus for tissue repair are important questions that need further investigation.

CONCLUSION

Considering the methodology used, the following may be concluded:

01. Calcium hydroxide associated with the saline or the iodoform plus saline showed antimicrobial effectiveness in both experimental methods. Analysis of the numerical data

TABLE 2- Antimicrobial effect of the pastes by direct exposure test

	S. aureus AGPC	E. faecalis AGPC	P. aeruginosa AGNR	B. subtilis AGPR*	C. albicans Yeast	Mixture e
CHS						
24	---	---	---	+++	---	---
48	---	---	---	+++	---	---
72	---	---	---	---	---	---
CHIS						
24	---	---	---	+++	---	---
48	---	---	---	---	---	---
72	---	---	---	---	---	---
IS						
24	---	---	+++	+++	---	+++
48	---	---	---	+++	---	+++
72	---	---	---	+++	---	+++

+++ presence of growth; --- absence of growth

(AGPC = aerobic Gram-positive coccus; AGNR = aerobic Gram-negative rods; AGPR* = aerobic Gram-positive rods, spore-forming)

showed no significant differences between both pastes containing calcium hydroxide, and significant differences with iodoform paste. The iodoform did not influence the antimicrobial potential of calcium hydroxide paste.

REFERENCES

- 1- Aydos JH, Milano NF. Revisão bibliográfica sobre o uso do Iodofórmio em Endodontia. *Rev Fac Odontol Porto Alegre*. 1984;26:43-51.
- 2- Bramante CM, Benatti-Neto C, Lia RCC, Esberard RM. Tratamento de perfurações radiculares com pastas de hidróxido de cálcio e iodofórmio – emprego de diferentes veículos – estudo em dentes de cães. *Rev Bras Odontol*. 1986;18:20-30.
- 3- Castagnola L. The use of iodoform paste (Walkhoff method) in modern endodontic therapy. *Quintessence Int*. 1976;7:19-23.
- 4- Castagnola L, Orlay HG. Treatment of grangene of the pulp by the Walkhoff method. *Br Dent J*. 1952;93:93-102.
- 5- Daniel RLDP, Jaeger MMM, Machado MEL. Emprego do iodofórmio em Endodontia – revisão da literatura. *Rev Pos-Grad*. 1999;6:175-9.
- 6- Estrela C. *Ciência endodôntica*. São Paulo: Artes Médicas; 2004.
- 7- Estrela C, Bammann LL, Pimenta FC, Pécora JD. Control of microorganism in vitro by calcium hydroxide pastes. *Int Endod J*. 2001;34:341-5
- 8- Estrela C, Bammann LL, Sydney GB, Moura J. Efeito antibacteriano de pastas de hidróxido de cálcio sobre bactérias aeróbias facultativas. *Rev Fac Odontol Bauru*. 1995;3:33-8.
- 9- Estrela C, Holland R. Calcium Hydroxide: study based on scientific evidences. *J Appl Oral Sci*. 2003;11:269-82.
- 10- Estrela C, Mamede Neto I, Estrela CRA, Pécora JD. Evaluation of density of calcium hydroxide pastes in dog's mandible. *Braz Endod J*. 1998;3:24-30.
- 11- Estrela C, Ribeiro RG, Estrela CRA, Pécora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine by different methods. *Braz Dent J*. 2003;14:58-62.
- 12- Estrela C, Sydney GB, Bammann LL, Felipe O Jr. Mechanism of the action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. *Braz Dent J*. 1995;6:85-90.
- 13- Evans M, Davies JK, Sundqvist G, Fidgor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *Int Endod J*. 2002;35:221-8.
- 14- Garcia-Godoy F. Evaluation of an iodoform paste in root canal therapy for infected primary teeth. *ASDC J Dent Child*. 1987;54:30-4.
- 15- Guedes-Pinto A, Paiva JG, Bozzola JR. Tratamento endodôntico de dentes decíduos com polpa mortificada. *Rev Ass Paul Dent*. 1981;35:240-5.
- 16- Holland R, Gonzales AC, Nery MJ, Souza V, Otoboni-Filho JA, Bernabé PFE. Efecto de los medicamentos colocados en el interior del conducto, hidrosolubles y no hidrosolubles en el proceso de reparación de dientes de perro con lesión periapical. *Endodoncia*. 1999;17:90-100.
- 17- Holland R, Maisto OA, Souza V, Maresca BM, Nery MJ. Acción y velocidad de reabsorción de conductos radiculares en el tejido conectivo periapical. *Rev Asoc Odontol Argentina*. 1981;69:7-17.
- 18- Maisto OA, Capurro MA. Obturación de conductos radiculares com hidróxido de cálcio-iodofórmio. *Rev Ass Odontol Argentina*. 1964;52:167-73.
- 19- Maisto OA, Erausquin J. Reacción de los tejidos periapicales del molar de la rata a las pastas de obturación, reabsorbibles. *Rev Ass Odontol Argentina*. 1965;53:12-20.
- 20- Maisto OA. *Endodoncia*. Buenos Aires: Mundi; 1967.
- 21- Matsuzaki K, Fujii H, Machida Y. Experimental study of pulpotomy with calcium hydroxide-iodoform paste in dog's immature permanent teeth. *Bull Tokyo Dent Coll* 1990;31:9-15.
- 22- Nair PNR. Pathobiology of the periapex. In: Cohen S, Burns RC. *Pathways of the pulp*. St. Louis: Mosby; 2002.
- 23- Pucci FM. *Conductos radiculares: anatomia, patologia y terapia*. Montevideo: Medico Quirurgica; 1945.
- 24- Siqueira Jr JF, Lopes HP, Magalhães FAC, Uzeda M. Atividade antibacteriana da pasta de hidróxido de cálcio/ paramonoclorofenol canforado/ glicerina contendo diferentes proporções de iodofórmio sobre bactérias anaeróbias estrictas e facultativas. *Rev Paul Odontol*. 1997;19:17-21.
- 25- Vojinovic O, Sрниé E. Induction of apical formation by the use of calcium hydroxide and iodoform – Chlumsky paste in the endodontic treatment of immature teeth. *J Br Endod Soc*. 1975;8:16-22.