Antimicrobial activity of 2% chlorhexidine gluconate, 1% sodium hypochlorite and paramonochlorophenol combined with furacin against S. aureus, C. albicans, E. faecalise and P. aureginosa

Atividade antimicrobiana da clorexidina a 2%, hipoclorito de sódio a 1% e paramonoclorofenol com furacin sobre S. aureus, C. albicans, E. faecalise e P. aureginosa

Tereza A. Delle Vedove Semenoff [a]
Alex Semenoff-Segundo [b]
Alvaro Henrique Borges [b]
Fabio Miranda Luiz Pedro [b]
Leonardo Stephan Caporossi [b]
Aurélio Rosa-Júnior [b]

[a] Graduate Program in Dentistry, UNESP, Araçatuba, SP, Brazil
[b] University of Cuiabá, Cuiabá, MT, Brazil

Abstract

Purpose: To analyze the antimicrobial action of 2% chlorhexidine gluconate, 1% sodium hypochlorite and paramonochlorophenol were combined with furacin against strains of *S. aureus, C. albicans, E. faecalis* and *P. aureginosa*.

Methods: Forty Petri plates were used. Four plates were separated from the others and used as a negative control. Four other plates were used a positive control. The other 32 plates were treated with four circles of sterilized filter paper impregnated with the test and control substances. The groups of plates were analyzed after an experiment time of seven days by the measuring the inhibition halos.

Results: Two percent chlorhexidine gave the largest inhibition zones, and the difference in size between its inhibition zones and the compound with the next largest inhibition zone, 1% sodium hypochlorite, was statistically significant. The 1% sodium hypochlorite also had significantly larger inhibition zones than the control group and PMC+F.

Conclusion: Two percent chlorhexidine gluconate gave the best results, while PMC+F showed the weakest antibacterial activity.

Key words: Chlorhexidine; sodium hypochlorite; Endodontics

Resumo

Objetivo: Analisar a ação antimicrobiana da clorexidina a 2%, do hipoclorito de sódio a 1% e do paramonoclorofenol associado ao furacin sobre *S aureus, C albicans, E faecalise* e *P aureginosa*.

Metodologia: Foram utilizadas 40 placas Petri. Quatro placas foram separadas como controle negativo e em 4 outras, além do meio de cultura, semearam-se os microrganismos, com o círculo de papel, para se obter o controle positivo. Em 32 placas seguiu-se a colocação de 4 círculos de filtro de papel esterilizados e impregnados das substâncias testes e controle, depositados em cada quadrante das mesmas. Os grupos foram analisados por 7 dias. Para a verificação dos resultados, usaram-se os halos de inibição de crescimento bacteriano.

Resultados: A clorexidina 2% foi significantemente ($P<0,05$) mais efetiva para todas as cepas microbianas que as demais substâncias. O hipoclorito de sódio a 1% apresentou resultados intermediários. O paramonoclorofenol associado ao furacin (PMC+F) obteve os piores resultados.

Conclusão: A clorexidina obteve os melhores resultados. O PMC+F apresentou os menores halos de inibição.

Palavras chave: Clorexidina; hipoclorito de sódio, Endodontia

Correspondence:
Tereza Aparecida Delle Vedove Semenoff
Rua professora Azélia Mamoré de Melo, 318/63
Cuiabá, MT – Brasil
78005 700
E-mail: t.semenoff@uol.com.br

Received: July 23, 2008
Accepted: November 18, 2009

Conflict of Interest Statement: The authors state that there are no financial and personal conflicts of interest that could have inappropriately influenced their work.

Introduction

It is the general consensus that the most important etiologic factor associated with the appearance and continuation of inflammatory periapical lesions is the presence of microorganisms in the root canal system (1-2). In this context, complete root canal system sanification is fundamental to the success of the endodontic treatment (3). Even with the great variety of available instrumentation techniques and irrigant solutions, it is still common to observe the presence of pulpar residues, bacteria and a smear layer after chemomechanical preparation (4). It is extremely important that the chemical solution used has a broad spectrum antimicrobial activity, is stable and effective against necrotic residues (5), can be easily inserted and removed from the inner root canal, and does not injure the pulp-periapical tissues (6).

Sodium hypochlorite is commonly used as an endodontic irrigant because of its excellent antimicrobial action (7-8) as well as its ability to clean dentinal walls and to facilitate tissue dissolution (9). However, these properties are related to the solution's concentration (10), and it is known that more concentrated solutions are less bio-compatible (11).

Chlorhexidine gluconate is better known for its excellent biocompatibility (13) than for its antimicrobial effectiveness (12). It has given excellent results associated with dental tissue and oral mucosa adhesion for a long period of time (14,15). This substance adheres to the cell wall of Gram positive and negative bacteria, causing selective protein precipitation from the cell wall, cytoplasm coagulation and the breakdown of intracellular components (12). This mechanism allows chlorhexidine gluconate to act as a bacteriostatic agent at low concentrations and a bactericide at high concentrations (14-16).

The combination of paramonochlorophenol and furacin has been proposed to try to reduce the irritant effects of the isolated phenolic compound, which are associated with camphor in periapical tissues (17). The antimicrobial effect occurs by the action of phenolate and chlorine ions that cause cell membrane permeability and protein denaturation, which affect the enzymes that are vital to the microorganism's survival (17,18).

Agar diffusion tests are widely used for the evaluation of antimicrobial activity (19). This method involves the placement of paper disks, which have been previously saturated with solutions of the agent in question, on Petri dishes containing agar inoculated with a selected microorganism. After a certain period of time, zones of inhibition of varying diameters form around the paper disks if the tested substance has antimicrobial activity (20).

Based on the above discussion, it should be expected that all three substances, even though they have different characteristics, will have a desirable antimicrobial effect. Thus, the objective of the study was to compare the antimicrobial activity of 2% chlorhexidine gluconate, paramonochlorophenol combined with furacin and 1% sodium hypochlorite against *S. aureus, C. albicans, E. faecalis* and *P. aureginosa.*

Methodology

Strains of S. *aureus, C. albicans, E. faecalis* and *P. aureginosa* were obtained from the Microbiology Department of the University of Cuiabá (UNIC), Cuiabá, MT, Brazil. Forty blood agar dishes were used. Thirty-two dishes were designated for the evaluation of the test substances. Four dishes were used to evaluate the growth of each microorganism and served as a positive control. Additionally, four dishes were used as a negative control and were not inoculated with any microorganism with the aim of evaluating of the absence of contamination in the respective culture mediums.

The microorganisms were inoculated into 7 mL of BHI (Brain Heart Infusion – Difco Laboratories, Detroit, Michigan, USA) broth and placed in an incubator for 24 hours at a constant temperature of 37 °C for replication. At the end of this phase, the cells should have a concentration close to $3x10^8$ cells/mL, which is similar to tube #1 on the McFarland scale.

Absorbent paper disks, obtained from patterned perforations of coffee filter paper were properly sterilized and moistened with each test solution (e.g., 2% chlorhexidine gluconate, 1% sodium hypochlorite and paramonochlorophenol combined with furacin), and one circle was moistened with 0.9% saline solution as a control. All the disks were immersed for an equal time in the respective solutions and neatly placed on sterile gauze to remove excess liquid.

After the immersion of the paper disks, they were observed at 12 h (the 0.9% saline solution), 15 h (the paramonochlorophenol combined with furacin), 18 h (the 1% sodium hypochlorite) or 21 h (the 2% gluconate chlorhexidine). After this observation, the plates were maintained in the incubator at 37 °C for 7 days.

The halos were measured in millimeters by a trained examiner using a stereoscopic magnifying scope and a millimeter ruler (Jon, Com. de Produtos Odontológicos, São Paulo, SP, Brasil). Data were analyzed using an ANOVA statistical test, which was conducted with a *Bonferoni* correctiontest at a 5% significance level.

Results

The inhibition halos for some of the antimicrobial agents had united, and they were excluded from the experiment. The results are described in Table 1.

Two percent chlorhexidine gluconate when compared to the other substances had halos that were larger by a statistically significant amount ($P<0.05$) than the other agents for all of the microorganisms. The 1% sodium hypochlorite was significantly less effective ($P<0.05$) than 2% chlorhexidine gluconate but more effective ($P<0.05$) than PMC combined with furacin and the control. PMC and control plates were not significantly different ($P>0.05$).

Table 1. Arithmetic means of the diameters (mm) of the bacterial inhibition halos for the various substances. Reported as the mean±standard deviation.

Solution Microorganism	PMC +Furacin	2% Chlorhexidine Gluconate	1% Sodium Hypochlorite	Saline
S. aureus	5.25±0.37 [c]	15.81±0.70 [a]	8.93±0.97 [b]	5.00±0.00 [c]
C. abicans	5.31±0.70 [c]	16.35±2.46 [a]	11.12±0.64 [b]	5.00±0.00 [c]
E. faecalis	5.12±0.35 [c]	14.18±3.18 [a]	8.68±0.88 [b]	5.00±0.00 [c]
P. aureginosa	5.37±0.28 [c]	13.00±0.96 [a]	5.87±0.24 [b]	5.00±0.00 [c]

* ANOVA and Bonferroni's tests ($P<0.05$). Different letters signify a statistically difference relative to the other groups.

Discussion

The objectives of chemomechanical preparation are pulpar residue removal, dentinal wall cleaning and the complete sanitation of the root canal system (3). In cases of acute abscess and in the absence of professional ability, the cleaning process is impossible to accomplish in the first session and it becomes necessary to use an intracanal dressing with antimicrobial activity (21).

The selection of sodium hypochlorite, chlorhexidine gluconate and paramonochlorophenol combined with furacin are based on the fact that these three substances are commonly used in acute abscess cases (22). Other substances sometimes used, such as tricresol formalin, act by vapor liberation, which could interfere with the microbial growth and halo formation of other substances (23). In addition, the dental class has restricted use because of its carcinogenic potential and cytotoxicity (24). To carry out the calcium hydroxide test, it would be necessary that the wells in the culture medium would be suitable for paste insertion, which would make it difficult to obtain the patterned measurement.

S. aureus, C. albicans, E. faecalis and *P. aureginosa* are extremely pathogenic and virulent as well as are important in the formation and organization of microflora in root canal systems (25). As virulence factors, these organisms have the capacity to neutralize immunoglobulins, lyse cells and tissues and easily invade human tissue (20). Previous literature demonstrates that chlorhexidine gluconate, sodium hypochlorite and PMC combined with furacin are effective antimicrobial agents for these microorganisms, although there are few studies that simultaneously compare the substances (6).

The results show that 2% chlorhexidine gluconate has the greatest inhibitory action, which is followed by 1% sodium hypochlorite, and is in agreement with other studies (7,15). With regard to PMC combined with furacin, slight antimicrobial activity was observed, but it was statistically similar to the negative control. This fact is controversial when compared to others results, which have demonstrated this substance's effectiveness (18).

While there are previous studies that evaluate the antimicrobial effect of chlorhexidine, sodium hypochlorite and PMC combined with furacin, we note that because of distinct methodologies it is difficult to compare the results of these studies and the antimicrobial performance of these substances (3,17,21). The size, the aqueous nature and uniformity of the inhibition halos can cause significant differences in methodologies of this nature (6). The 2% chlorhexidine gluconate solution gave a larger uniform inhibition halo when compared to the other substances. Because the absorbent paper disks had the same diameter and were moistened in a similar manner, it could be that the slight loss of surface tension from the chlorhexidine facilitated the spreading of the compound in a uniform manner.

Conclusions

This *in vitro* study showed that the antimicrobial activity of 2% chlorhexidine gluconate was greater than the other substances examined. A 1% sodium hypochlorite solution was the second most effective, while PMC combined with furacin was not effective against the microorganisms tested.

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.
2. Nair RN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod 1990;16:580-8.
3. Siqueira JF Jr, Batista MM, Fraga RC, Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. J Endod 1998;24: 414-6.
4. Yesilsoy C, Whitaker E, Cleveland D, Phillips E, Trope M. Antimicrobial and toxic effects of established and potential root canal irrigants. J Endod 1995;21:513-5.

5. Siqueira Jr JF. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J 2001;34:1-10.
6. Estrela C, Ribeiro, RG, Estrela, CR, Pécora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. Braz Dent J 2003;14: 58-62.
7. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2,5% sodium hypochlorite and 0,2% chlorexidine guconate separately and combined endodontic. J Endod 1998;24:472-76.
8. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55:307-12.
9. Grossman L, Meiman B. Solution of pulp tissue by chemical agents. J Am Dent Assoc 1941;28:223-5.
10. Morse DR. Endodontic microbiology in the 1970s. Int Endod J 1981;14:69-79.
11. Pashley E, Birdsong N, Bowman K, Pashley D. Cytotoxic effects of NaOCl on vital tissue. J Endod 1985;11:525-8.
12. Fardak O, Turnbull R. A review of literature on use of chlorhexidine in dentistry. J Am Dent Assoc 1985;112:863-9.
13. Greenstein G, Berman C, Chlorhexidine JR. An adjunct to periodontal therapy. J Periodontol 1986;57:370-6.
14. Leonardo MR, Filho MT, Silva LA, Filho N. *In vivo* antimicrobial activity of 2% chlorhexidine used as root canal irriganting solution. J Endod 1999;25:167-71.
15. White RR, Hays GL, Janer IR. Residual antimicrobial activity after canal Irrigation with chlorhexidine. J Endod 1997;23:229-31.
16. Dametto FR, Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:768-72.
17. Estrela C. Eficácia antimicrobiana de pastas de hidróxido de cálcio. [Thesis]. Ribeirão Preto (SP): Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo; 1997.
18. Holland R. Emprego tópico de medicamentos no interior dos canais radiculares. Odonto Master: Endod 1994;1:1-13.
19. Sassone LM, Fidel RA, Murad CF, Fidel SR, Hirata Jr R. Antimicrobial activity of sodium hypochlorite and chlorhexidine by two different tests. Aust Endod J 2008;34:19-24.
20. Tobias RS. Antibacterial properties of dental restorative materials: a review. Int Endod J 1988;21:155-60.
21. Lynne RE, Liewehr FR, West LA, Patton WR, Buxton TB, Mcpherson JC. In vitro antimicrobial activity of various medication preparations on *E. faecalis* in root canal dentin. J Endod 2003;3:187-92.
22. Vier-Pelissera FV, Garbina RP, Só MVR, Marca C, Pelissera A. Medicações intracanal e sistêmica preconizadas nas faculdades de Odontologia brasileiras para o tratamento de urgência do abscesso periapical agudo. Rev Odonto Ciênc 2008;23:278-2.
23. Melo AB, Albuquerque DS, Castro CMB. Estudo comparativo in vitro da capacidade antimicrobiana das diluições do tricresol formalina. JBE 2004;5:126-31.
24. Thomas MI, Gonçalves TS, Souza MA, Schmitt VM. Formaldeído na odontologia: aspectos antimicrobianos, carcinogênicos e mutagênicos. Um estudo da sua viabilidade na clínica odontológica. Rev Odonto Ciênc 2006;21:387-91.
25. Estrela, C, Bammann LI, Pimenta FC, Pecora JD. Control of microorganisms in vitro by calcium hydroxide pastes. Int Endod J 2001;34:341-5.