Antimicrobial Action of a Filling Paste Used in Pulp Therapy in Primary Teeth under Different Storage Conditions.

Juliana Rodrigues Praetzel* / Fabiana Vargas Ferreira** / Rita Niederauer Weiss *** /
Rosane Salete Friedrich **** / Antonio Carlos Guedes-Pinto *****

Objective: *compare the antimicrobial effectiveness of common antimicrobial paste immediately after pulp therapy and in different storage conditions.* ***Study design:*** *Staphylococcus aureus, Staphylococcus epidermis, Streptococcus mutans, Streptococcus oralis, Enterococcus faecalis; Escherichia coli and Bacillus subtilis sample were used. The storage conditions utilized were: at room temperature - the paste was subjected to a natural aging process at room temperature for a 24-hour and 7, 14, 28, 60 and 90 day periods (GPRT). Refrigerator storage – the paste was placed to a natural aging process in a refrigerator at 4°C for a 24 hour and 7.14, 28, 60 and 90 day periods (GPR). To perform the immediate effect analysis (GPi or zero time), of each mixture either at room or refrigerator temperature, the preparation procedure was similar, although GPR, iodoform, Rifocort® and camphorated paramonochlorophenol were stored in the refrigerator and then taken out, dosed and manipulated, and had their immediate effect tested after a l-week period at storage of 4°C average temperature. The testing methodology was Dilution in Solid Medium-Agar. The Wilcoxon Test and Friedman variance analyses were used.* ***Results:*** *the GP paste showed antimicrobial activity at all experimental times.* ***Conclusion:*** *the pastes presented antimicrobial effectiveness at all experimental times.*
Keywords: *Primary teeth, root canal obturation, pulp, endodontics,*
J Clin Pediatr Dent 33(2): 27–30, 2008

INTRODUCTION

A filling paste proposed by Guedes-Pinto *et al*[1] composed of iodoform, camphorated paramonochlorophenol and Rifocort® is used for endodontic treatment of primary teeth by over 90% of all Dentistry Schools in Brazil in pulpectomy cases (45,5% pure or 45,45% associated with ZOE) and by over 40% in biopulpectomy cases (25% pure and 16,60% associated with ZOE).[2] The successful association of these three drugs in the endodontic therapy of primary teeth has been proven by several studies.[3,4,5,6,7,8,9,10]

Pulp therapy of primary teeth is a treatment that demands rather long treatment sessions with the child is sometimes being unwilling to cooperate.[7] When the paste proposed by Guedes-Pinto is used, its preparation can enhance this unwillingness to cooperate. However, if this paste was previously prepared for use, there would be a significant contribution in decreasing preparation time. Thus, this paper evaluated the immediate antimicrobial activity of the Guedes-Pinto paste[1] as well as the antimicrobial activity to different storage conditions and different times.

STUDY DESIGN

The antimicrobial activity was tested of the paste against microorganisms found in root systems. To approximate these canals, *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermis* (ATCC 11228), *Streptococcus mutans* (ATCC 25175), *Streptococcus oralis* (ATCC 10557) and *Enterococcus faecalis* (ATCC 29212) samples were used; an *Escherichia coli* (ATCC 25922) sample was used; and, a *Bacillus subtilis* (ATCC 6633) sample was used.

The paste was prepared in accordance with Guedes-Pinto *et al.* who established standards (1981) from a mixture of three pharmaceutical products at the following rate: 0,30g iodoform; 0,1 ml camphorated paramonochlorophenol (PMCC) (3:7) and 0,25g Rifocort®. For the current study, the paste was referred to as GP.

* Juliana Rodrigues Praetzel, DDS, MSD, PhD, Adjunct Professor - School of Dentistry - Department of Stomatology, Federal University of Santa Maria, Rio Grande do Sul, Brazil.
** Fabiana Vargas Ferreira, DDS, MS Master Degree Student, Dental Science Post-Graduate Program, Pediatric Dentistry, Department of Stomatology, School of Dentistry, Federal University of Santa Maria, Rio Grande do Sul, Brazil.
*** Rita Niederauer Weiss, Member of Department of Microbiology, Federal University of Santa Maria, Rio Grande do Sul, Brazil.
**** Rosane Salete Friedrich, Member of Department of Microbiology, Federal University of Santa Maria, Rio Grande do Sul, Brazil.
***** Antonio Carlos Guedes-Pinto, DDS, MSD, PhD, Chairman Professor, School of Dentistry, Department of Pedodontics, USP, Sãn Paulo University, Brazil.

Send all correspondence to: Fabiana Vargas Ferreira, Rua Visconde de Pelotas, 517, CEP 97010-440. Santa Maria, Rio Grande do Sul, Brazil.

Phone: (55) 3 225 24 35
(55) 3 91 61 11 89

E-mail: fabivfer@yahoo.com.br

To study the antimicrobial activity of the stored paste, the following storage conditions were provided: storage at room temperature - the paste was submitted to a natural aging process at room temperature for a 24-hour and 7, 14, 28, 60 and 90 day periods and refrigerator storage – the paste was submitted to a natural aging process in a refrigerator at 4°C for a 24 hour and 7, 14, 28, 60 and 90 day periods for the mixed paste.

To perform the immediate effect analysis (GPi or zero time), either at room or refrigerator storage temperature, the preparation procedure was similar, although GPR, iodoform, Rifocort® and PMCC were stored in the refrigerator and taken out, dosed, manipulated, and had their immediate effect tested after a l-week storage at 4°C average temperature. The abbreviation used for these paste varieties were GPiRT and GPiR, respectively.

The immediate evaluation of the antimicrocial activity was performed soon after drug manipulation, taking zero time into account; the study of the stored paste antimicrobial effect was performed by analyses of GPRT and GPR at the previously established experimental 24-hour and 7, 14, 28, 60 and 90-day intervals.

The testing method used in the current study was Dilution in Solid Medium - Agar in plaque with modified (BHI) + Agar 2% + 5% defibrinated sheep blood (for *Streptococcus mutans* and *Streptococcus oralis*).[11]

Control plates were used to confirm bacterial growth in the 'pour plate', without the addition of the test paste, at 37°C for a 24 – 48 hour period.

Bacteriostatic activity was verified by measuring the antimicrobial growth inhibition bands.[11] In order to verify bactericidal activity for each period, an inhibition band section of each paste being tested was removed with a bacterial loop and added to a nutrient broth for a 24/48-hour period for bacterial growth verification, which was detected by the appearance of an opacity in the middle section of the test specimen. If such opacity did not occur, the bactericidal action of the paste on the microorganisms under analysis was confirmed; in the case of bacterial growth, the test organism was confirmed by nutrient agar replication. All analyses were duplicated and carried out under aseptic conditions in a laminar flow hood.

Descriptive analysis followed by non-parametrical statistics was applied. The Wilcoxon Test was used to compare the two storage procedures for each test microorganism at each experimental time. Friedman variance analysis was employed to test the behavior of each test microorganism at each experimental time and in each storage procedure as well as to evaluate differences among experimental times for each storage procedure of each individual microorganism separately.

RESULTS

All results in this study were considered at a 5% significance level.

Descriptive statistics of the data referring to averages of growth-inhibition bands of test microorganisms is shown in Table 1.

GP paste showed antimicrobial activity against all microorganisms tested at all experimental times both at room and refrigerator temperature, as follows:

— GP paste proved to be bacteriostatic for *Staphylococcus mutans*; *Streptococcus oralis*; *Staphylococcus aureus; Staphylococcus epidermis, Escherichia coli; Enterococcus faecalis and Bacillus subtilis;*

— GP paste proved bactericidal for: *Streptococcus mutans; Streptococcus oralis; Staphilococcus aureus; Staphilococcus epidermis* and *Escherichia coli*; it did not present bactericidal action on *Enterococcus faecalis* and *Bacillus subtilis*.

The results for nonparametric statistics applied to this study showed that there were no statistically significant differences among the different experimental periods of time for all test microorganisms when the paste was stored at room temperature (Friedman Variance Analysis Chi Sq.: 5,849582; p< 0,44027) or refrigerator temperature (Friedman Variance Analysis – Chi Sq: 8,745206; p < 0,18846); also, there were no statistically significant differences for *Streptococcus mutans* (p = 0,498967); *Streptococcus oralis* (p = 0,600183); *Staphylococcus aureus* (p = 0,799848); *Staphylococcus epidermis* (p = 0,3455455); *Escherichia coli* (p = 0,067898); *Enterococcus faecalis* (p = 0,753154) and *Bacillus subtilis* (p = 0,079625) at all experimental times with regards to the paste storage procedure (Wilcoxon); additionally there were no statistically significant differences among experimental times for *Streptococcus mutans* (Chi Sqr.: 5,886793; p < 0,43601); *Streptococcus oralis* (Chi Sq.: 11,60377; p < 0,07145); *Staphylococcus aureus* (Chi Sq.: 3,196262; p < 0,78384); *Staphilococcus epidermis* (Chi Sq.: 6,000000; p < 0,42321); *Escherichia Coli* (Chi Sq.: 9,962264; p < 0,12629); *Enterococcus faecalis* (Chi Sqr.: 4,046512; p < 0,67038) and *Bacillus subtilis* (Chi Sq.: 8,679611; p < 0,19245) for each storage procedure (Friedman Variance Analysis).

DISCUSSION

The success of an endodontic treatment depends on many factors with the reduction or elimination of bacterial infection, being the most important one. The patient's favorable response and to the optimal removal of necrotic tissue from the canal system. It also depends on the effective action of the drugs used to disinfect the canals.[12] Therefore it is necessary to associate an appropriate technique with a low cytotoxic material, which must present excellent antimicrobial properties against microorganisms usually found in contaminated canals.[10] Chemical substances used in pulp therapy in primary teeth must present a good tissue tolerance, and be able to resorb physiologically,[12] and must have excellent antiseptic properties associated with its ability to diffuse into the dentinal tubules.[14]

The agar diffusion method which was used in the present study is one of the most commonly employed to evaluate

Table 1. GP Paste antimicrobial activity descriptive statistics for each test microorganism in the two storage procedures.

Descriptive statistics (mm)	Value of n	Average	Median	Minimum	Maximum	1st Quartile	3rd Quartile	Standard Deviation
RTSmutans	7	9,714286	10,0	8,0	12,0	8,0	11,0	1,496026
Rsmutans	7	10,17857	10,5	9,0	11,5	9,5	10,5	0,825559
RTSoralis	7	7,964286	7,0	6,3	10,5	6,5	9,5	1,673498
RSoralis	7	7,821429	7,0	5,8	10,0	6,0	10,0	2,075222
RTEfaecalis	7	5,142857	5,0	5,0	5,5	5,0	5,5	0,243975
REfaecalis	7	5,214286	5,0	3,0	6,5	5,0	6,0	1,149534
RTSaureus	7	5,75	5,5	4,5	8,0	5,0	6,3	1,163687
RSaureus	7	5,392857	5,8	4,0	6,0	4,5	6,0	0,814672
RTEcoli	7	7,285714	7,0	6,0	10,0	6,0	8,5	1,523624
REcoli	7	6,5	6,0	5,5	8,5	5,5	7,5	1,118034
RTSepidermidis	7	9,285714	9,0	6,5	14,0	7,5	10,0	2,395432
RSepidermidis	7	10,82143	9,5	8,3	21,0	8,5	10,0	4,547697
RTBsubtilis	7	19,28571	18,0	12,5	24,0	16,0	24,0	4,706885
RBsubtilis	7	21,85714	23,0	19,0	24,0	19,5	24,0	2,392946

RT = room temperature / R = refrigerator temperature

antimicrobial activity.[6,7,10,14] However, some peculiarities should be taken into consideration when applying this method: the weight, size, and molecular shape of the antimicrobial agent; the agar gel texture; the loading and concentration of the tested material and the contact between the tested material and the agar.[15]

The microorganisms used in the current study were selected because they are found commonly in infected root canals of primary teeth.[3,6,7,10] Additionally, microorganisms serve as a reference in quality control procedures used in antimicrobial sensitivity tests, such as *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus*[16] while *Bacillus subtilis* presents some resistance to sterilization, chemical and physical processes.[17]

A medication used against a microorganism is measured by the bacteriostatic and bactericidal properties; these properties, in turn, depend on the toxicity of the substance to the type of microorganism under consideration and on the dispersion of the substance.[11] In order to attain optimum antimicrobial action, an association of the two factors is desirable since bacterial growth restarts the moment a bacteriostatic agent is removed. The results obtained in this study showed the GP paste had a favorable antimicrobial action along with an exceptional diffusion capability against all test microorganisms, in agreement with other studies.[3,6,7,8,9,10] Inhibition effectiveness in all paste versions, regardless of storage procedure times, resulted in microbial growth inhibition bands whose average values varied between 3mm and 24mm (Table 1). Although the susceptibility of each microorganism to the paste was different, the bacteriostatic antimicrobial effect persisted throughout the experiment, with no relevant individual variations found. Also, significant differences among experimental times for each test microorganism in either storage procedure were not observed.

As the band width is directly proportional to the inhibition action of the test agent, it can be inferred from the results obtained that the antimicrobial action of GP occurred in decreasing order against: *Bacillus subtilis, Streptococcus oralis, Streptococcus mutans, Staphylococcus epidermis, Escherichia coli, Staphylococcus aureus* and *Enterococcus faecalis.*

The evidence for the last three bacteria corroborates current literature that attests to the strong resistance of these microorganisms to antimicrobial agents to the extent that these microorganisms are used as quality control referrals in procedures applied to their sensitivity tests.[7,10,16] However, the current data differ from those obtained in other studies,[6] where GP paste also showed bactericidal action against *Enterococcus faecalis*.

The methodology used in this research was different and the agent diffusion was not tested on a microorganism pour-plate but on Agar, similar to the Disc Diffusion Method. Additionally, the proportion of drugs from the original paste was changed until a 'toothpaste consistency' was achieved, which may have altered the antimicrobial action of the paste.

Bonow[3] studied the original antimicrobial action of the GP paste as well as that of the modified paste by means of alterations in its components, with the addition of calcium hydroxide (CH), Zinc Oxide, or both (the so-called mixed paste) and found a sequential antimicrobial effect decrease as follows: paste with the addition of Zinc Oxide, mixed paste, and paste with CH addition.

The effect of low temperatures upon microorganisms depends on the specific microbe and the intensity of application. At average refrigerator temperatures (0 to 7°C), the metabolic rate of some organisms is reduced to the extent that they cannot reproduce or synthesize toxins. Regular cooling has bacteriostatic effect.[18] However, this research found that, regardless of the studied storage conditions, the refrigerated paste showed an effect similar to that for the paste at room temperature, and this property was maintained throughout the experiment.

The bacteriostatic effect of low temperatures was not observed, probably because the cooled paste, when used, had its effect tested in a dry heat sterilizer at 37°C.

The results obtained by statistical analyses showed that the storage procedure, independently of the period of time tested, did not interfere with the paste antimicrobial activity

when the microorganisms were analyzed either individually or in a group. This finding suggests that the paste can be prepared and stored both at room temperature and under refrigeration for daily use in clinics without affecting its antimicrobial effectiveness. Santos[4] and Souza *et al*.[5] compared the *in vitro* and *in vivo* paste cytotoxicity, along with other drugs used in endodontic therapy of primary teeth and found that the GP paste was the most biocompatible and, therefore, the least cytotoxic, of all. The Toxicity found by Santos (4) was attributed to the presence of PMCC in the formula.

The antimicrobial action found in GP paste seems to be related to its components,[12] Iodoform has a germicidal effect for some authors.[3,6] For authors, iodoform only acts bacteriostatically when in contact with organic secretions or infected areas *in vivo*,[18,19] such as those in the root canal system. PMCC is an antimicrobial agent that has an effective bacteriostatic action, either topically or systemically[3]; with this characteristic is related to its concentration. Rifocort® is a product formed from a corticosteroid and an antibiotic, presenting a great antimicrobial action and recommended for the treatment of primary teeth presenting pulpal infectious processes.

Enterococcus faecalis was considered the most resistant organism in relation to the antimicrobial effectiveness. This resistance has already been reported in many studies[7,10,16] and can be responsible for failure in the successful endodontic treatment of primary teeth.[1] This microorganism can be present in the oral cavity in children, mainly those who regularly use a contaminated pacifier.[20] *Enterococcus faecalis* can be found in the oral cavity flora, through contamination, and is part of the microbiota of infected canals of primary teeth.[7,13] and invade the pulp and dental tubules of teeth presenting pulpite and necrosis.13 Therefore, it seems difficult to prevent the presence of *Enterococcus faecalis* in the root canals of those teeth that bear lesions and which are exposed to the oral cavity.

The need for routine clinical and radiographic control to check whether the paste is been resorbed from inside the treated canal systems is worth mentioning, as well as the remission of any pre-existing pathological processes or the absence of new alterations.

With reference to the bacteriostatic effect of the test paste against *Bacillus subtilis*, it offers no risks inasmuch as this microorganism does not participate in the oral pathogenic flora. It was only included in this study as a quality parameter for the sensitivity test of an antimicrobial agent, having exhibitied an excellent action.

CONCLUSION

The Guedes-Pinto paste[1] showed bacteriostatic action against all test organisms in the two storage procedures and at all experimental times (24 h, 7, 14, 28, 60 and 90 day periods); the paste also presented bactericidal action against most organisms except for *Enterococcus faecalis* and *Bacillus subtilis*.

REFERENCES

1. Guedes-Pinto, AC.; Paiva, JG.; Bozzola, JR. Tratamento endodôntico de dentes decíduos com polpa mortificada. Rev Assoc Paul Cir Dent, 35: 240–45, 1981.
2. Costa, MEPR.; Chianca, T.; Ferreira, FT.; Charlier, S.; Bastos, MSCO. O ensino das diferentes técnicas de terapia pulpar em Odontopediatria. Pesqui Odontol Bras, 14: 104, 2000.
3. Bonow, MLM. Atividade antimicrobiana de drogas usadas na terapia pulpar de dentes decíduos. (Dissertação de Mestrado) – Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, p. 56, 1993.
4. Santos, EM. Análise da citotoxicidade in vitro de fármacos utilizados na terapia endodôntica de dentes decíduos: estudo comparativo da ação da pasta Guedes-Pinto, formocresol, glutaraldeído e ácido fosfórico sobre cultura de fibroblastos. (Dissertação de Mestrado) - Faculdade de Odontologia, Universidade de São Paulo, São Paulo, p. 115, 1998.
5. Souza, CJA.; Lacativa, AM.; Pascon, EA.; Loyola, AM. Avaliação histológica da resposta óssea a pasta Guedes-Pinto: estudo experimental em cobaias. Pesqui Odontol Bras, 14: 131, 2000.
6. Silva, CM.; Candelária, LFA.; Bombana, AC. Estudo comparativo da ação antimicrobiana entre cinco pastas de obturação de canais de dentes decíduos. J Bras Odontopediatr Odontol Bebe, 5: 502–510, 2002.
7. Praetzel, JR. Atividade Antimicrobiana da Pasta Guedes-Pinto Imediata e Armazenada Em Condições Diversas Por Diferentes Períodos de Tempo. (Tese de Doutorado) – Faculdade de Odontologia, Universidade de São Paulo, p. 116, 2003.
8. Amorim, LFG de; Toledo, OA de; Estrela, CRA de; Decurcio, DA de; Estrela, C. Antimicrobial analysis of different root canal filling pastes used in Pediatric Dentistry by two experimental methods. Braz Dent J, 17: 317–322, 2006.
9. Massa, VTDM; Brum, SC; Oliveira, RS; Carvalho, CA; Ferraz, CA; Alves, UM et al. Avaliação da atividade antimicrobiana da pasta Guedes-Pinto, em diferentes tempos de armazenagem. Disponível em: [http://www.sbpqo.org.br/resumos/2006; IC2]. 2006
10. Ferreira, FV. Atividade antimicrobiana da pasta Guedes-Pinto associada à clorexidina [Monografia de Conclusão de Curso]. Santa Maria: Faculdade de Odontologia, Universidade Federal de Santa Maria, RS, p. 40, 2007.
11. Romão, CMCA; Miyazaki, NHT; Motta, LLF. Métodos para Análise Microbiológica de Saneantes com Ação Antimicrobiana. In: Manual de saneantes – Instituto Nacional de Controle de Qualidade em Saúde. Rio de Janeiro: Fiocruz, pp. 33–36, 1992.
12. Guedes-Pinto, AC. Tratamento endodôntico em dentes decíduos. In: Odontopediatria, Guedes-Pinto, A.C. São Paulo: Santos, pp. 535–555, 1997.
13. Godoy, VL. Distribuição de bactérias planctônicas, colônias bacterianas e biofilmes microbianos em dentes decíduos com pulpite e ou necrose pulpar. (Tese de Doutorado) - Faculdade de odontologia de Bauru, Universidade de São Paulo, Bauru, p. 151, 1999.
14. Tchaou, WS; Turng, BF; Minah, GE; Coll, JA. In vitro inhibition of bacteria from root canals of primary teeth by various dental materials. Pediatr Dent, 17: 351–55, 1995.
15. Tanomaru, JMG; Pappen, FG; Tanomaru Filho, M; Spolidorio, DMP; Ito, IY. In vitro antimicrobial activity of different gutta-percha points and calcium hydroxide pastes. Braz Oral Res, 21: 35–9, 2007.
16. Oplustil, CP; Zoccoli, CM; Tobouti, NR; Sinto, SI. Testes de avaliação da resistência aos antimicrobianos. In: Oplustil, CP; Zoccoli, CM: Tobout, NR; Sinto, SI. Procedimentos básicos em microbiologia clínica. São Paulo: Sarvier, 165–180, 2000.
17. Spicher, G.; Peters, J. Suitability of *Bacillus subtilis* and *Bacillus stearthermophilus* spores as test organism bioindicators for detecting superheating of steam. Zentralbl Hyg Umweltmed, 199: 462–474, 1997.
18. Tortora, GJ.; Funke, BR.; Case, CL. Controle de crescimento bacteriano. 6 ed. In: Microbiologia. Porto Alegre: Artes Médicas Sul, 181–206, 2000.
19. Pucci, FM. Conductos radiculares: anatomia, patología y terapia. Buenos Aires: Médico Quirúrgica, 343–395, 1945.
20. Tomasi, E., Victora, CG., Barros, FC., Olinta, MT. Uso de chupeta em crianças: contaminação fecal e associação com diarréia. In: Congresso Brasileiro de Epidemiologia, 2, 1990, Belo Horizonte. Programa e Resumos, 1990, 173.