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ESTUDO COMPARATIVO DA ATIVIDADE ANTIMICROBIANA "*IN VIVO*" DE SEIS ANTISSÉPTICOS BUCAIS SOBRE A MICROBIOTA DA SALIVA*

A comparative *in vitro* study of antimicrobial activity of six commercial mouthwashes against salivary bacteria

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Resumo

Introdução: Soluções de antissépticos bucais podem desempenhar importante papel no controle químico do biofilme dentário. No entanto, os procedimentos de controle de qualidade relacionados com a atividade antimicrobiana destes enxaguatórios contra bactérias da cavidade oral não são bem divulgados. **Objetivo:** Avaliar a atividade antimicrobiana in vivo de seis soluções de antissépticos bucais disponíveis no mercado brasileiro, empregadas como enxaguatórios contra bactérias da saliva humana. **Material e métodos:** Um estudo in vivo foi desenvolvido com indivíduos voluntários (8 do sexo masculino e 7 do sexo feminino, variando de 18 a 63 anos de idade), independente do estado de saúde bucal. Os seguintes produtos comerciais foram testados durante 2 horas após um único procedimento de bochecho: 1) Plax®, 2) Listerine®, 3) Periogard®, 4) Cepacol®, 5) Sanifill Premium® e 6) Oral B®.Os resultados foram analisados pelo teste de ANOVA de medidas repetidas e ANOVA one-way com um nível de significância de 5%. **Resultados:** Houve diferença significativa ($p < 0,05$) observada na diminuição da carga microbiana para Plax® entre o início (antes anti-séptico bucal) e imediatamente após o bochecho (T0); para Periogard® entre os valores iniciais e T60 (60 minutos após o bochecho), na linha de base e

T120 (120 minutos após o bochecho) e B® Oral entre os valores iniciais e T-30 (30 minutos após o bochecho). Periogard® apresentou a maior redução da carga microbiana salivares. **Conclusão:** Dos seis bochechos testados, Plax®, Oral B® e Periogard ® apresentou atividade antibacteriana imediata. Periogard® foi o anti-séptico bucal que mostrou a atividade mais prolongada contra bactérias anaeróbias salivares.
Palavras-chave: Antissépticos Bucais; Saliva; Bactérias.

Abstract

Introduction: Mouthwashes solutions can play an important role in the chemical control of dental biofilm. However, quality control procedures related to antimicrobial activity of these solutions against oral bacteria are not well known. **Objective:** To evaluate *in vivo* antimicrobial activity of six mouthwashes solutions available in the Brazilian market against anaerobic salivary bacteria. **Material and methods:** An *in vivo* study was developed in human volunteers (8 male and 7 female, ranging from 18 to 63 years old), despite their oral health status. The following commercial products were tested after 2 hours of a single mouthwash procedure: 1) *Plax*®, 2) *Listerine*®, 3) *Periogard*®, 4) *Cepacol*®, 5) *Sanifill Premium*® and 6)*Oral B*®. Data were analyzed by ANOVA to repeated measures and ANOVA *one-way* with a significance level of 5%. **Results:** Statistically significant difference ($p<0.05$) was observed in the decrease of microbial counts to *Plax*® between baseline (before mouthwash) and immediately after mouthwash (T0); to *Periogard*® between baseline and T60 (60 minutes after mouthwash), baseline and T120 (120 minutes after mouthwash) and to *Oral B*® between baseline and T-30 (30 minutes after mouthwash). *Periogard*® showed the highest and delayed reduction of salivary microbial counts. **Conclusion:** Out of six tested mouthwashes, *Plax*®, *Oral B*® and *Periogard* ® showed immediate antibacterial activity. *Periogard*® was the oral anti-septic that showed the best delayed activity against salivary anaerobic bacteria.
Keywords: Mouthwashes; Saliva; Bacteria.

Introduction

Mouthwashes solutions, along with toothpastes can play an important role in the chemical control of dental biofilm, as well as therapeutic agents against caries, gingivitis and halitosis[4,5,8]. Many formulations were developed containing various antimicrobial agents such as sodium fluoride, quaternary ammonium compounds (cetylpyridinium chloride), phenols (triclosan), essential oils (menthol, thymol, eucalyptol), natural herbal products (sanguinarine) and bisguanide (chlorhexidine)[4]. Nowadays, it is quite evident the great expansion of these commercial products in worldwide market. According to ABIHPEC[1] (Brazilian Association of Personal Hygiene and Cosmetics Industry), the Brazilian market of oral hygiene product is growing significantly and perspective is to reach R$2,45billions by 2012. In Brazilian market, the production and distribution of mouthwashes solutions are controlled by a national agency of the Department of Health (Agência Nacional de Vigilância Sanitária – ANVISA). Despite the direct application of these solutions on mouth and potential risk of absorption on oral mucosa and ingestion, the agency classifies commercial mouthwashes in the same category of cosmetics, deodorants, perfumes and other products for personal hygiene[2].Thus, tests related to safety and efficacy (antimicrobial activity in oral cavity) are not required with same rigidness as for other therapeutic agents (antibiotics, vitamins, for example). This study was developed with the purpose to evaluate the antimicrobial activity against salivary anaerobic bacteria of some commercial mouthwashes solutions available in Brazilian market.

Material and Methods

This short-time longitudinal study was approved by the Ethical Comitee of "Instituto de Estudos de Saúde Coletiva da Universidade Federal do Rio de Janeiro"- process # 49/2008. Six commercial oral mouthwashes (table 1) were tested *in vivo* in a double blind independent study involving 15 volunteers (8 males and 7 females, ranging from 18 to 63 years old), despite their oral health status.

Table 1. Brands, manufactures and antimicrobial agents of the mouthwashes tested.

Mouthwashes	Manufacturer	Antimicrobial agents
Plax®	Colgate – Palmolive Company São Paulo, SP , Brasil	Triclosan(0,03%), sodium fluoride (0,05%)
Listerine®	Johnson & Johnson do Brasil São José dos Campos, SP, Brasil	Eucalyptol (0,092%), Thymol(0,064%), Menthol(0,042%), Methyl salicilate (0,06%)
PerioGard®	Colgate -Palmolive Company São Paulo, SP, Brasil	Chlorhexidine digluconate (0,12%)
Cepacol®	Sanofi Aventis Farmaceutica Ltda , São Paulo, SP, Brasil	Cetylpyridinium chloride (0,05%) Menthol*, Eucalyptol*, Methyl salicilate*
Sanifill Premium®	Facilit Odontologica e Perfumaria Ltda , Rio de Janeiro, RJ, Brasil	Polyhexametilene biguanide (0,35%). Sodium fluoride (0,05%)
Oral B®	Procter & Gamble do Brasil São Paulo, SP, Brasil	Cetylpyridinium chloride* Sodium fluoride*

*Concentration not informed by the manufacturer

The ability of these products to reduce the concentration of salivary anaerobic bacterial counts was tested in a period of 2 hours after a single mouthwash procedure. All the volunteers were informed about the objective and methodology of the study and asked to sign a compliance form of participation with explanations. Each volunteer was submitted in different days (48 hours of wash out) to a mouthwash with 10 milliliters during one minute of the following commercial products: 1) Plax®, 2) Listerine®, 3) Periogard®, 4) Cepacol®, 5) Sanifill Premium®, 6)Oral B®. Stimulated salivary samples were taken in the following times: baseline (immediately before mouthwash procedure), T0 (immediately after mouthwash), T30, T60, T120 (30, 60 and 120 minutes after mouthwash, respectively). In order to cover most of putative oral pathogens, all salivary samples were diluted in ten fold and 0,1mL of 10-3 and 10-4 dilutions and plated on blood agar and incubated in GasPak® anaerobic jar and anaerobic generator with catalyst at 37°C during 48 hours for further determination of colony forming units per mL of saliva (cfu/mL). Numbers of cfu/mL were transformed in Log10 to statistical analysis.

Statistical analysis

After Log10 transformation of cfu /mL numbers, for most of the time searched, there was a normal distribution for each of mouthwash solution tested. ANOVA to repeated measures was applied to verify the differences among the ufc counts during the five periods studied for each commercial product. Comparison between two different periods was accomplished by paired t test with Bonferroni penalty. ANOVA *one-way* with *Bonferroni* post-test was applied to verify differences among mouthwashes in each period of test. Significance level of all tests was 5%.

Results

When we analyzed each mouthwash separately during period of assay, it was observed significant decrease of microbial counts to *Plax®, Periogard® and Oral B®*. In relation to *Plax®,* the reduction *was observed* between baseline and T0; *Periogard®,* between baseline and T60 and baseline and T120; *Oral B®* between baseline and T30. In addition, we observe that *PerioGard®* revealed the highest and most delayed reduction of salivary microbial counts (table 2).

Table 2. Mean ± sd (Log 10) of cfu salivary anaerobic bacteria counts in different periods of assay.

Mouthwashes	Periods	Mean ± SD	*p*
Plax®	Baseline [a]	7,41 ± 0,76	0,001
	T0 [b]	6,73 ± 0,72	
	T30 [a,b,]	7,09 ± 0,60	
	T60 [a,b]	7,09 ± 0,55	
	T120 [a,b]	7,22 ± 0,53	
Listerine®	Baseline	7,41 ± 0,85	0,462
	T0	7,17 ± 1,09	
	T30	7,24 ± 0,70	
	T60	7,13 ± 0,72	
	T120	7,35 ± 0,80	
PerioGard®	Baseline [a]	7,39 ± 0,73	0,004
	T0 [a,b]	6,63 ± 0,85	
	T30 [a,b]	6,92 ± 0,67	
	T60 [b]	6,80 ± 0,61	
	T120 [b]	6,82 ± 0,63	
Cepacol®	Baseline	7,42 ± 0,89	0,057
	T0	6,96 ± 0,83	
	T30	7,15 ± 0,68	
	T60	7,37 ± 0,60	
	T120	7,12 ± 0,76	
Sanifill Premium®	Baseline	7,79 ± 0,63	0,209
	T0	7,32 ± 0,66	
	T30	7,44 ± 0,53	
	T60	7,51 ± 0,38	
	T120	7,32 ± 0,79	
Oral B®	Baseline [a]	7,60 ± 0,50	0,01
	T0 [a,b]	7,19 ± 0,60	
	T30 [b]	7,30 ± 0,35	
	T60 [a,b]	7,40 ± 0,32	
	T120 [a,b]	7,40 ± 0,31	

Same letters show no significant difference, when comparing two periods.

In the comparison of all mouthwashes in each studied period, *Periogard®* showed significant difference at 60 minutes after rinses in relation to *Sanifill Premium®* and *Oral B®* (table 3).

Table 3. Mean ± sd (Log 10) of cfu salivary anaerobic bacteria counts to each period of assay considering all mouthwashes.

Periods	Mouthwashes	Mean ± SD	*p*
Baseline	*Plax®*	7,41±0,76	0,425
	Listerine®	7,41±0,85	
	Periogard®	7,39±0,73	
	Cepacol®	7,42±0,89	
	Sanifill Premium®	7.79±0,63	
	Oral B®	7,60±0,80	
T0	*Plax®*	6,77±0,72	0,107
	Listerine®	7,17±1,09	
	Periogard®	6,63±0,85	
	Cepacol®	6,96±0,83	
	Sanifill Premium®	7,32±0,66	
	Oral B®	7,19±0,60	
T30	*Plax®*	7,09±0,60	0,228
	Listerine®	7,24±0,70	
	Periogard®	6,92±0,67	
	Cepacol®	7,15±0,68	
	Sanifill Premium®	7.44±0,53	
	Oral B® [a, c]	7,30±0,35	
T60	*Plax®* [a, c]	7,09±0,55	0,014
	Listerine® [a, c]	7,13±0,72	
	Periogard® [a]	6,80±0,61	
	Cepacol® [a, c]	7,37±0,60	
	Sanifill Premium® [b,c]	7,51±0,38	
	Oral B® [b, c]	7,40±0,32	
T120	*Plax®*	7,22±0,53	0,144
	Listerine®	7,35±0,80	
	Periogard®	6,82±0,63	
	Cepacol®	7,12±0,76	
	Sanifill Premium®	7,32±0,79	
	Oral B®	7,40±0,31	

Same letters show no significant difference, when comparing two mouthwashes.

Discussion

Chemical reduction of the dental biofilm is an objective followed by researchers and industry of oral chemical agents for many years. Many agents were developed, but none of them is able to reduce specifically some bacteria related to oral diseases. Several investigations in this subject are sponsored by industries and the results published in journals where mouthwashes are largely advertised. Therefore, independent studies in this field are necessary.

In this sense, this independent *in vivo* study was developed aiming to evaluate the reduction of total salivary anaerobic bacteria during two hours after rinses. The design of the study shows information on the substantivity of the active agents of the major mouthwashes marketed in Brazil. This property, that reflects the ability of an antiseptic to inhibit bacterial growth in the oral environment, is directly related to its delayed release after adsorption to glycoproteins recovering dental and mucosal surfaces[7]. Thus we believe that the results found reflect absence, low or high substantivity of the antimicrobial components of the mouthwashes tested.

The best delayed activity of *Periogard*® against salivary microorganisms can be explained by chlorhexidine content. According to Schiott, et al[10], chlorhexidine once adsorbed in the oral cavity shows a persistent bacteriostatic action for 12 hours. Although an *in vitro* study has shown that association between chlorhexidine and sodium fluoride has no beneficial effect because decreasing substantivity of chlorhexidine[3], an *in vivo* investigation showed efficacy of the combination in the reduction of *Streptococcus mutans* levels when compared with chlorhexidine and sodium fluoride alone[6]. Certainly, salivary glycoproteins develop a fundamental role in the results found in the *in vivo* studies as shown in our investigation. We believe that short period of antimicrobial activity found in *Plax*® and *Oral B*® was due to the combination of *Triclosan*® *and Gantrez*® in *Plax*® and the content of cetylpyridinium chloride in *Oral B*®*.* On the other hand, this antimicrobial activity was not observed in *Cepacol*® probably because the cetylpyridinium chloride content is lower or the combination with other components can reduce its antimicrobial activity. In *Plax*®, this combination showed no increased activity in antimicrobial activity against salivary microorganisms when compared to that found with *Periogard*®*.*

The combination *of* polyhexametilene biguanide and sodium fluoride in *Sanifill Premium*® revealed no benefit for delayed antimicrobial activity*.*

The total absence of significant antimicrobial activity found to *Listerine*® and *Cepacol*® can be explained by low substantivity of their contents. However, Pan, et al.[9] employing *in vitro* static and flow-through biofilm systems,in a not independent study, concluded that essential oil showed superior antiplaque biofilm activity to amine and stannous fluoride, cetylpiridine chloride/chlorhexidine and cetylpiridine chloride and comparable activity to chlorhexidine.

Nevertheless, despite of some favorable results obtained in both *in vivo* and *in vitro* studies with mouthrinses, concerns with their safety related to shifts on the oral microbial ecosystem should be pointed out. As suggested by Gunsolley, et al.[4] the clinical benefits of antiplaque and anti-gingivitis mouthrinses are similar to those of oral prophylaxis and oral hygiene instructions at six months recall appointments.

Conclusions

Plax® reduced salivary anaerobic microorganisms just immediately after rinse, while *Oral b*® *and Periogard*® reduced them in 30 and 30 and 60 minutes, respectively.

For all mouthwashes, for each period tested, difference was observed 60 minutes after rinses between *Periogard*® *and Sanifill Premium*® and *Periogard*® *and Oral B*®.

Periogard® was the oral antiseptic that showed the best delayed activity against salivary anaerobic microorganisms.

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