

IN VITRO ANTIBACTERIAL ACTIVITY OF SILVER DIAMINE FLUORIDE IN DIFFERENT CONCENTRATIONS

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ABSTRACT

The antibacterial activity of Silver Diamine Fluoride – SDF – (Cariestop®), at commercial concentrations of 12% and 30%, was evaluated against clinical and pattern strains (ATCC 25175) of *S. mutans*. Clinical isolates were obtained from the saliva of six children attending the Pediatric Dentistry Clinic UFPB, being grouped as follows: GI- low risk and caries activity, GII- high risk and caries activity. Once sown, the strains were isolated from Mitis-Salivarius Agar and divided into seven groups - M1 (pattern strain) to M7. The antibacterial activity was determined by maximum inhibitory dilution (MID) by the agar diffusion method, using serial dilutions (1:1 to 1:32) and the pure formulations of SDF and chlorhexidine 0.12% (positive control). After incubation, the inhibition zones were measured. The bactericidal and bacteriostatic actions of pure substances and in their respective MIDs were evaluated by test of germicidal power, using glass specimens, after inoculation by the strains and incubation for 24

hours in BHI broth. Each specimen was exposed to chlorhexidine and to SDF for 30s, 3min, 30min and 1h, then incubated for 24 h in BHI broth. The samples were subcultured in Mitis-Salivarius Agar to evaluate the bactericidal or bacteriostatic activity of the substances. The data were analyzed in a comparative-descriptive method. The Cariestop® 30% was effective until the last dilution (1:32) on all strains. For Cariestop® 12%, the MIDs corresponded to last dilution (1:32) in almost all samples, except for M3 (1:8). Chlorhexidine showed DIM in the last concentrations (1:32) on five samples, and in the concentration 1:8 for M3 and M7. As evidence of the germicidal power, the substances had bactericidal activity at all times analyzed. It was concluded that the cariostatic showed antibacterial activity when compared to chlorhexidine and these two substances presented bactericidal action against the strains at all contact times.

Key words: cariostatic agents, anti-bacterial agents, dental caries.

ATIVIDADE ANTIBACTERIANA IN VITRO DO DIAMINO FLUORETO DE PRATA EM DIFERENTES CONCENTRAÇÕES

RESUMO

Avaliou-se a ação antibacteriana do Diamino Fluoreto de Prata (Cariestop®) nas concentrações comerciais de 12% e 30% frente às linhagens clínicas de *Streptococos* do Grupo Mutans (EGM) e padrão (ATCC 25175) de *S. mutans*. Os isolados clínicos foram obtidos da saliva de seis crianças atendidas na Clínica de Odontopediatria da UFPB, sendo agrupados em: GI-baixo risco/atividade de cárie; GII-alto risco/atividade de cárie. Após semeadura, as cepas foram isoladas em Agar Mitis-Salivarius e divididas em sete grupos - M1 (linhagem padrão) a M7. Determinou-se a ação antibacteriana por meio da Diluição Inibitória Máxima (DIM) pelo método da difusão em ágar, utilizando-se diluições seriadas (1:1 a 1:32) das formulações puras dos cariostáticos e da clorexidina a 0,12% (controle positivo). Após incubação, mensurou-se os halos de inibição. A ação bactericida e bacteriostática, das substâncias puras e nas suas respectivas DIMs, foi avaliada pela prova do poder germicida, utilizando-se corpos de provas de vidro, inoculados pelas cepas e incubados por 24h em meio BHI. Cada corpo

de prova foi exposto aos cariostáticos e à clorexidina por 30s, 3min, 30min e 1h então inseridos em caldo BHI e incubados por 24h. As amostras foram repicadas em Agar Mitis-Salivarius para avaliação da ação bactericida ou bacteriostática das substâncias. A análise dos dados foi comparativo-descritiva. O Cariestop® a 30% foi eficaz até a última diluição (1:32), frente todas as cepas. Para o Cariestop® a 12%, as DIMs corresponderam à última diluição (1:32) em todas as amostras, exceto para M3 (1:8). A clorexidina apresentou DIM na última concentração (1:32) em cinco amostras, sendo 1:8 para M3 e M7. A prova do poder germicida identificou ação bactericida das substâncias nos tempos analisados, para todas as amostras. Concluiu-se que os cariostáticos apresentaram ação antibacteriana quando comparados à clorexidina e estas duas substâncias exerceram ação bactericida frente às cepas, em todos os tempos de contato.

Palavras chaves: agentes cariostáticos, agentes antibacterianos, cárie dentária.

INTRODUCTION

Dental caries have multi-factor origins. The most outstanding one is the interaction between the dental biofilm, a saccharose-rich diet and a host prone to acquiring such condition¹. There are several methods for caries prevention. The most popular

ones are the control of biofilm combined with a healthy diet and the regular use of fluorides. Yet, even if such practices are increased, there has been a 60% prevalence of caries in five-year old children, according to the data from the SB Project in Brazil. On the other hand, for toddlers between 18 and 36

months, an average of 27% of these individuals presented at least one deciduous element with caries². Thus, the prevention and control of dental caries must be undertaken as early as in the first years of age to avoid the condition as well as to minimize its progression in cases where it has already been detected. Within this context, it is important to use agents that control the progression of lesions: while waiting for treatment or for the adaptation of children to new hygiene and nourishing habits³.

Among control agents, we would like to underscore the action of silver diamine fluoride⁴, as a cariostatic and preventive agent. Its main effect on dental structures is the promotion of formation calcium fluoride and silver phosphate, without any loss of phosphate and calcium ions⁵. Silver diamine fluoride is found in 10%, 12%, 30%, and 38% concentrations³. It is an easy-to-apply colorless liquid, hence, no technical devices are needed for its application. The antibacterial *in vitro* action of the cariostatic agent was observed by Montadon, Sperança⁶. According to the authors, this product acts on *S. mutans*, so, with higher concentrations, higher effects. The use of silver diamine fluoride in *in vivo* assays has already been mentioned in literature⁷⁻¹².

According to Rosenblatt, Stamford and Niederman⁴, there is evidence that silver diamide fluoride is more effective than fluoride varnish in preventing caries lesions and stopping their development, which fact enhances the capability of the preventive action of this material. These authors also point out that this preventive agent seems to meet the criteria of both the World Health Organization and of the American Medical Institute of medical care in the XXI century. Considering this and the quest for new results that prove the bactericide and bacteriostatic action of Silver Diamine Fluoride (SDF), the purpose of this research was to assess, *in vitro*, the antibacterial action of such agent in different concentrations, on *Streptococcus mutans* (ATCC 25175) and of Streptococci of the Mutans group (SMG) isolated in the oral cavity.

MATERIALS AND METHODS

The antibacterial action of silver diamine fluoride (Cariestop®), commercially available in 12% and 30% concentrations, was studied on *S. mutans* ATCC 25175 and on SMG clinical isolates. For this purpose, saliva of buccal mucosa was collected through buccal swab from six patients of the *Pediatric Dentistry Clinic* of the Federal University of Paraíba, Brazil. Patients were divided into two groups (GI and GII), according to the

risk and the activity of caries, measured by the Simplified Oral Hygiene Index - OHI-S¹³, by the Gingival Bleeding Index - GBI¹⁴ and def-t. Insertion was made on active white spot lesions, diagnosed through visual exam of the surfaces of duly cleaned and dried enamel, considered to be opaque and rough, thus, visually different from translucent enamel^{15,16}.

In group GI, the agent was inserted in individuals with low-risk of caries and low caries activity, with a 0 to 1.0 SOHI, and a GBI lower than 10%, with no actual presence of caries, through diagnosis of white spot lesions and active caries. Inclusion in group GII was done in a population with a 2.0 to 3.0 SOHI, a GBI higher than 10% and with presence of caries and active white spot lesions. Saliva specimens and clinical trials were taken by a single investigator. This study was approved by the Research Ethics Committee of the Center of Health Sciences of the Universidade Federal de Paraíba (UFPB), Clinical Trial N° 0594. The inclusion of children was authorized by the responsible parties through signature of a Free and Informed Consent.

After collection of the biological material in Agar Mitis-Salivarius, as per technical practices proposed by Gold, Jordan, Van Houte¹⁷, clinical strains of SMGs were isolated. Thus, six clinical strains were obtained, which were labelled as M2 to M8, M1 being the standard strain of *S. mutans* (ATCC 25175). The clinical specimens were identified as Streptococci of the Mutans Group by using Agar Mitis -Salivarius. Additionally, a catalase test and a Gram stain test were undertaken¹⁸.

Antibacterial activity was measured through the Maximum Inhibitory Dilution technique (MID Technique). The diffusion agar assay was used, using the well diffusion plate method. Six serial dilutions of the cariostatic agent were obtained, in sterile distilled water, as well as of the control solution (chlorhexidine 0.12%). Additionally, pure concentrations of all the products were used. The MID was considered the greatest dilution capable of promoting the formation of inhibition halos. Antibacterial activity of the cariostatic agents was classified as bacteriostatic or as bactericidal according to the methodology proposed by Sperança and Teles¹⁹, with alterations, using the germicide power test. Hence, five different contact periods were selected: thirty seconds, three minutes, thirty minutes, one hour, and twenty-four hours.

Thus, 120 test specimens were used in glass specimens (6 mm diameter), for doing the germicide power test, which was done both on the MIDs and on the pure forms of the cariostatic agents at 12% and 30%, as well as on chlorhexidine at 0.12%. After identifying

Table 1: Values of serial dilutions in MIDs of substances in *S. mutans* specimens.

| Substances | Assessed Strains | | | | | | |
|---------------------|------------------|------|------|------|------|------|------|
| | M1 | M2 | M3 | M4 | M5 | M6 | M7 |
| Cariestop® 12% | 1:32 | 1:32 | 1:8 | 1:32 | 1:32 | 1:32 | 1:32 |
| Cariestop® 30% | 1:32 | 1:32 | 1:32 | 1:32 | 1:32 | 1:32 | 1:32 |
| Chlorhexidine 0.12% | 1:32 | 1:32 | 1:8 | 1:32 | 1:32 | 1:32 | 1:8 |

the SMGs, three morphologically similar colonies were withdrawn from each Petri dish, which were dispensed into sterile test tubes with a BHI broth medium. Immediately after, they were incubated by the candle method at 37°C, for 24 hours. Subsequently, suspensions were compared as per the MacFarland scale (10^8 microorganisms /mL) and adjusted. The same procedure was followed for the *S. mutans* standard strain until seven specimens were completed for the germicide power test. Immediately after this, the specimens were incubated during 24 hours in incubator, in tubes containing 400µL of BHI, plus 100µL of bacterial inoculant. Then, the specimens were dispensed into tubes with 500µL of the products being tested, both in their MID and in their pure forms, and were exposed to the solutions for periods of thirty seconds, three minutes, thirty minutes, and one hour.

Once the contact periods were over, the specimens were withdrawn and then inserted into tubes with BHI medium broth and incubated in incubator during 24 hours. After the contact test, aliquots of specimens were replicated in agar Mitis-Salivarius¹⁷ with a Dri-galski Loop and compared to the growth control, consequently, in this group, no specimens were exposed to any substance test.

The analysis of data was done through descriptions, and the MID was the main dilution capable of producing inhibition halos. Bacteriostatic activity was characterized when a decrease in bacterial growth was observed, by comparison between the Colony Forming Units of the growth control and those subjected to the contact test. Then, bactericide activity was determined when there was no bacterial growth.

RESULTS

Silver diamine fluoride at 12% and 30% showed antibacterial activity in all the assessed specimens. The MIDs results for all the strains were diverse as

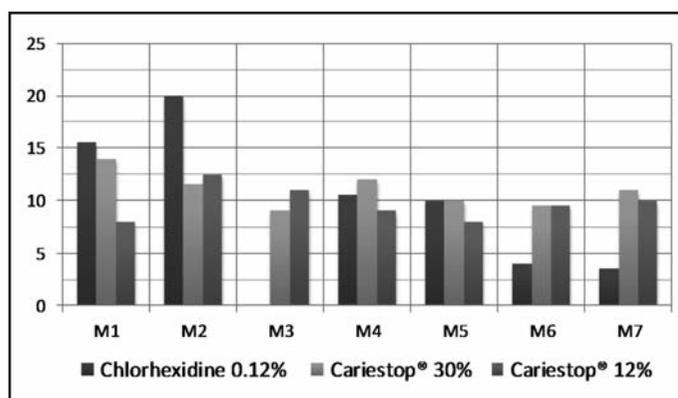


Fig. 1: Average values of inhibition halos (in mm) of substances in MIDs against *S. mutans* and SMG specimens.

a series of average values for inhibition halos were obtained (Fig. 1). Similarly, the control group (chlorhexidine at 0.12%) showed an anti-bacterial effect on the assessed specimens (Table 1).

As to the germicide power trial, it could be stated that Cariestop® at 12% and 30% had an effective bactericide activity in a 30-second minimum period when compared to the control group. It was confirmed that Cariestop® did not show any bacteriostatic activity in any of the periods as all the specimens were plaqued, and no bacterial growth was observed in any of the periods under analysis.

DISCUSSION

Silver Diamine Fluoride (SDF) solutions in 12% and 30% concentrations, and chlorhexidine, expressed antibacterial activity in all the strains that were analysed, with different inhibition halos for the different concentrations. As regards the standard strain (M1), the MID of the cariostatic agents and chlorhexidine were observed in the last dilution, that is, at 1:32. Yet, the average values of the inhibition halos were dissimilar. In this study it was observed that with the standard strain, the antibacterial activity of the cariostatic agents was more effective with greater concentrations, according to Montadon and Sperança⁶ who, when comparing

three SDF concentrations (10%, 12% and 30%), *in vitro*, stated that the higher the concentration of the solution, the higher the antimicrobial efficacy would be. Yet, with Alves²⁰ using the same methodology as described for the measuring of MID, it is stated that the activity of SDF at 12% was effective only in its pure formulation, and, consequently, the solution at 30% had antimicrobial activity up to the second dilution (1:2), with *S. mutans*. Thus, it is suggested that, in spite of the use of Cariestop® at 12% and 30% in both assays, the different sensitivities of the strains could be caused by the methodological criteria in place, such as the making of the bacterial suspensions and the cultivation of the specimens in a medium poor in nutrients.

As to the clinical isolates, it was observed that cariostatic agents and chlorhexidine had similar activity to microorganisms. It was demonstrated that Cariestop® at 30% in pure formulation had average inhibition halo values that were higher than with Cariestop® at 12%. Yet, when average halo values of MIDs are compared, no large discrepancies are verified.

As to Cariestop® at 12%, average values of halos were 19.0 mm for M4 and 22.5 mm for M6 and M7, in their pure form. In the case of MIDs, values moved from 8.0 mm to 12.5 mm for M5 and M2, respectively. It was observed that only in the M3 specimen, the MID did not correspond to the last dilution (1:32) because it was verified at 1:8, with an inhibition halo average value of 11.0 mm.

Even though in Montadon and Sperança⁶, the diameter of the inhibition halos is not mentioned, it is stated by the authors that the difference in the antibacterial potential between cariostatic agents is directly related to the initial concentration. This has also been observed in this research as, on average, the inhibition halos produced by Cariestop 30% were superior than those in the concentrate at 12%. Thus, considering the results under analysis, it seems feasible to suggest that the isolated specimens of the patients in GI and GII showed similar sensitivity levels to cariostatic agents, so much so that the present experience of caries and of SOHI and GBI values seem not to interfere with the sensitivity level of the clinical specimens of SMGs to cariostatic agents.

In the literature of reference, no other research was found with similar results, yet, other sources have demonstrated the cariostatic and antimicrobial action of silver diamine fluoride through *in vivo* assays^{4,7-12}. Consequently, a link between the results of this research and of those mentioned above could be deter-

mined, as all of them have aimed at assessing sensitivity levels of *S. mutans* to SDF, despite different methodologies used in each research. Hence, we confirm the antimicrobial action of SDF in 12% and 30% concentrations, on SMG clinical strain, based on these methodological criteria and results. These results are in line with those obtained by Medeiros et al.⁷; Collina, Moreira and Barbosa¹¹ and Almeida et al.¹².

It must be underscored that this research confirms the antimicrobial action of SDF, represented by Cariestop® at 12% and 30%. Yet, it is suggested that more research should still be undertaken, and that larger specimens of microorganisms and products of other commercial brands and concentrations should be used. Additionally, clinical assays could be combined with laboratory tests to verify if the behaviour of Silver Diamine Fluoride under laboratory conditions is similar to that verified under clinical conditions.

In the germicide power test it was verified that the action of cariostatic agents was essentially a bactericide action, as well as that of chlorhexidine. No difference was detected between activity of Cariestop® at 12% and at 30%.

Both the standard strain and clinical strains had the same reaction to the action of cariostatics as the different strains did not show evidence of growth after the germicide test. It was observed that timing was not a restriction to the bactericide action of cariostatic agents and of positive control, so much so that, after thirty seconds of contact between specimen and substances, no bacterial growth was identified. According to observations by Montadon and Sperança⁶, during a germicide power test of four strains of *S. mutans*, with periods of thirty seconds, one minute and three minutes, it was verified that SDF 30% had bactericide action on all the microorganisms. Thus, according to the findings of the authors mentioned above, in this research it was verified that SDF 30% showed its bactericide action on the strains, in all the periods proposed.

According to the methodology proposed by Sperança and Teles¹⁹, no desorption procedure was undertaken after the contact test because the methodology did not show any need for such procedure as it was assumed that periods of thirty seconds, three minutes, thirty minutes, and one hour would not be long enough for adhesion of this microorganism on a surface.

Another point to be underscored is the absence of saccharose in the BHI medium in which the specimen was immersed. This was a relevant methodological stage in the adhesion tests of *S. mutans* on glass sur-

faces, but needed not to be completed in this assay as the objective of this assay was to assess the germicide power by means of the contact test, and this stage was subsequent to the relevant assay.

Still in comparison with the assay of the reference, a difference was observed when using Cariestop® 12% because our results show that the product had a bactericide effect on the strains in all the intervals under analysis. Yet, according to the reference by Montadon and Sperança⁶, SDF 12% had a bactericide effect after three minutes for only one strain; then, the bacteriostatic action was verified for the other strains in all the time intervals in the research. In the bibliography that was consulted, no assays were found that would approach germicide power tests in clinical strains. Yet, we may suggest that, in the strays assessed, SDF in the concentrations of the assay is a bactericide agent. Taking into account the restrictions of laboratory tests as regards the use of clinical strains

in *in vitro* tests, we may suggest that the use of SDF affects the progression of dental caries. Considering what has been mentioned before, we suggest doing clinical tests, which will verify if the results obtained in this research take place in *in vivo* environments.

Considering the results hereby presented, it was concluded that after determining the MID, Silver Diamide Fluoride at 12% and 30% showed antimicrobial action compared to chlorhexidine at 0.12%. Additionally, by this germicide power test, cariostatic agents showed a bactericide effect on the strains under analysis, in the proposed time intervals, with no sensitivity-based differences between clinical strains and standard strains. There was evidence that Cariestop® 30% compared to 12%, both in their pure forms, had greater antibactericide action. On the other hand, it was determined that the antibacterial effect of chlorhexidine 0.12%, as determined by MIDs, was effective on all the strains, vis a vis cariostatic agents.

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