Effects of four anticaries agents on lesion depth progression in an in vitro caries model

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Abstract

Purpose: *This study compares four chemotherapeutic regimens used for inhibiting carious lesion progression: silver nitrate ($AgNO_3$); silver fluoride/stannous fluoride (AgF/SnF_2); silver diammine fluoride (SDF); and chlorhexidine (CHX).*

Methods: *For this study, a bacterial model system containing Mutans streptococci (MS) and Lactobacilli casei (L) was used to generate carious lesions on 85 extracted sound permanent third molars which were randomly assigned to four test groups and one control group. At week two, the four treatment regimens were applied to the lesions (one treatment per test group).*

Results: *Six weeks later, lesions treated with a single AgF/SnF_2 or $AgNO_3$ application demonstrated 29% and 19% less lesion progression, respectively, than did the control group ($P<0.05$). SDF and CHX did not differ significantly from the control.*

Conclusion: *AgF/SnF_2 and $AgNO_3$ may be useful in slowing down carious lesion depth progression. (Pediatr Dent 21:176–180, 1999)*

The past several years have seen renewed interest in minimal intervention techniques for the management of dental caries in children. This has stemmed from the realization that certain populations of children lack access to dental care.[1] Programs using Atraumatic Restorative Treatment (ART) have been implemented where more traditional dental treatment is not available.[2] In the US, such techniques were favored due to difficulties faced when treating early childhood caries (ECC) in very young children. Techniques employed in these cases can range from treatment of incipient carious lesions with fluoride varnish[3] to removal of caries with hand instruments and restoring the teeth with glass ionomer (ART).[2]

The concept of minimal intervention for the management of dental caries is not new. The use of chemical agents to slow down or arrest caries progression without surgical removal of the lesion has been documented in the literature for many years. A variety of agents have been used for this purpose including silver nitrate, silver fluoride/stannous fluoride, silver diammine fluoride, and chlorhexidine.

The use of silver nitrate ($AgNO_3$) to arrest caries progression has been described since early in this century.[4] Through the years, its use has fallen in and out of popularity and contradictory results regarding its efficacy in arresting caries have been reported.[5–7] It is now considered obsolete, but reference to its application can be found as recently as 1981.[8]

Silver diammine fluoride (SDF) has been used for the same purpose with references first appearing in 1969.[9] SDF offers several potential advantages over AgF. It forms minimally soluble CaF_2 in the enamel layer, the silver ion has bactericidal properties, and it is not caustic because it lacks the nitrate group. Several studies in Japanese children claim its effectiveness,[10–13] and at least one reference cites its use by dentists in the United States.[14]

In Australia, a silver fluoride/stannous fluoride combination (AgF/SnF_2) has been advocated for treatment of carious lesions in children.[15]

Chlorhexidine (CHX), available in gels, varnishes, mouthrinses, and dentifrices, has been widely utilized in dentistry since 1972 as an antiplaque, antimicrobial, antifungal, and antiviral agent. It shows promise as a cariostatic agent, since it has the ability to significantly reduce levels of oral MS. Several studies, mainly those using highly concentrated CHX varnishes, have shown that elimination of MS in adult subjects with high levels of MS is possible over relatively long periods of time.[16–20]

A number of studies have evaluated the effectiveness of these potentially cariostatic agents to reduce salivary MS counts, to alter the composition of the dental plaque flora, or to reduce the incidence or progression of caries. However, no comparison of these agents to each other has previously appeared in the dental literature. The purpose of this study was to compare the effectiveness of these four known treatment regimens in reducing carious lesion progression in an in vitro bacterial caries model.

Methods

Eighty-five extracted, permanent human upper third molars with sound approximal surfaces were used in this study. After soft-tissue removal and cleaning with a pumice slurry, the teeth were longitudinally cut in half in a buccolingual direction. Small holes were drilled near the root apices, and orthodontic ligature wire was threaded through these holes to allow for grouping, labeling (color coded for blinding), and suspension of specimens in the artificial caries medium. Each specimen was entirely covered with an acid resistant varnish (nail polish) except for an area of 1.5 x 6 mm on the approximal side, oriented with its longer dimension in mesiodistal direction. The prepared tooth halves were sterilized using ethylene oxide at 108°F.

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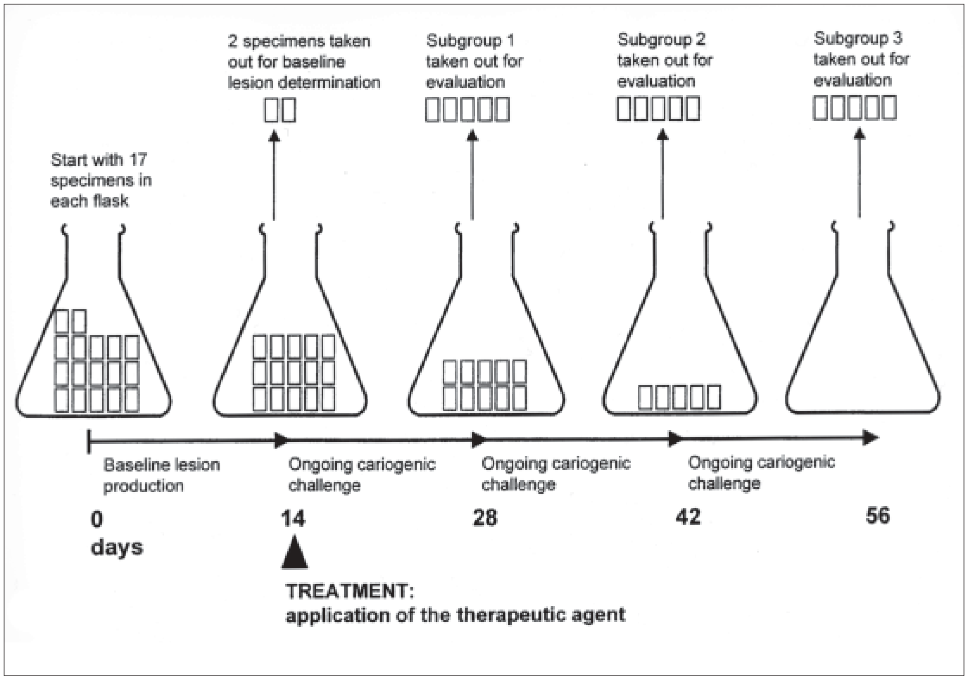

Fig 1. Study design.

The specimens were then aseptically transferred into five 225 mL flasks (17 per flask) containing Trypticase Soy Broth-Yeast Extract (TSB-YE) supplemented with 1% glucose and 2% sucrose. Primary cultures of MS and Lactobacillus casei PB 359 (L) were grown individually from frozen stock in TSB-YE in 5% CO_2 at 37°C for 18 hours. Flasks containing the medium and teeth were inoculated with 0.5 mL of each primary culture and then incubated for 48 hours in 5% CO_2 at 37°C. After 48 hours the teeth were transferred to new media and inoculated with fresh primary cultures. This incubation and transfer cycle was repeated every 48 hours during the eight-week study period to maintain a constant cariogenic challenge involving high concentrations of MS and L as well as frequent exposure to fermentable carbohydrates.

All specimens were initially treated in this manner for 14 days in order to create carious lesions which could then be treated with the agents. At day 14, two specimens from each flask were removed for determination of baseline lesion depth. The remaining 15 teeth in each flask were then randomly assigned to the following groups (one treatment per flask):

Group 1 ($AgNO_3$): a drop of ammoniacal silver nitrate (HOWE's solution) was applied to each lesion, followed by a drop of eugenol to reduce and precipitate the silver.

Group 2 (SDF): silver diammine fluoride 38% (Fluoroplat®) was applied to the dried surface for 3 min using a cotton pellet in a gentle rubbing manner.

Group 3 (AgF/SnF_2): silver fluoride 40% solution (AGF Silver fluoride®) was applied for 60 sec with a cotton pellet to the dry surface, followed by a layer of 10% stannous fluoride spot application paste (Floran®) flowed over the treatment site.

Group 4 (CHX): chlorhexidine gel (5% in 2.5% methylhydroxyethylcellulose) was applied to the dried surface for 3 min and the excess gently washed away with sterile water.

Group 5 (control group): isotonic saline was applied for 60 sec with a gentle rubbing manner on the dried surface.

At two-week intervals, five teeth from each treatment group were removed and evaluated for lesion progression. The study design (Fig 1) allowed lesion progression to be studied over a six-week period. Specimens were sectioned (100–120 μm thickness) using a Silverstone-Taylor Hard Tissue Microtome (Series 1000 Deluxe, Sci Fab, Littleton, CO). Four sections were taken from each specimen. The samples were imbibed in water, observed using polarized light microscopy and photographed using an Olympus Photomicrographic System PM10 AD. Slides were projected onto a table and traced onto paper. Lesion depth for each specimen was determined by taking the mean of five measurements in each section's tracing and calculating the mean of the four sections' mean.

Statistical evaluation considered two variables: lesion depth among the groups at each time period and lesion depth within each group over time. After determining that there was no interaction between groups and time (two-way ANOVA), a one-way ANOVA was performed with Duncan's Multiple Range post-tests to determine significant differences between groups. Values of $P<0.05$ were considered significant.

Results

The average baseline lesion depth determined from specimens of all groups was 237 μm (SD 22 μm). Lesion depths at two-, four-, and six-week intervals and the results of Duncan's Multiple Range Tests are presented in Table 1. The ANOVA test for significant differences between groups at all three time intervals revealed highly significant differences ($P<0.0002$). Fig 2 displays incremental lesion progression for each of the three time intervals. Fig 3 shows that at two and four weeks following treatment, the silver and fluoride containing agents were effective in slowing down the carious process, compared to the control group and the CHX group. Six weeks following treatment, the lesion depth progression remained significantly less for the AgF/SnF_2 and $AgNO_3$ groups than for the control group

Table 1. Total Lesion Depth in Microns (SD)

Treatment Group	2 Weeks Post-tx	4 Weeks Post-tx	6 Weeks Post-tx
AgF/SnF_2	276 (41) A	513 (29) A	714 (62) A
$AgNO_3$	320 (47) A,B	487 (69) A,B	778 (52) A
SDF	339 (35) B	576 (36) B	897 (42) B
CHX	410 (52) C	678 (91) C	913 (66) B
Control	429 (37) C	690 (26) C	906 (82) B

Numbers at the same treatment time with the same letter are not significantly different from each other. Others are significantly different at $P<0.05$.

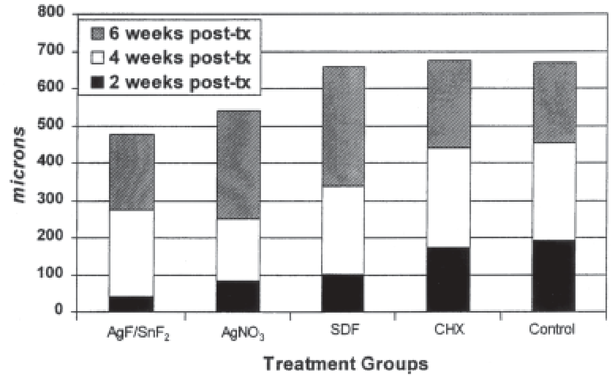

Fig 2. Increase in lesion depth over time.

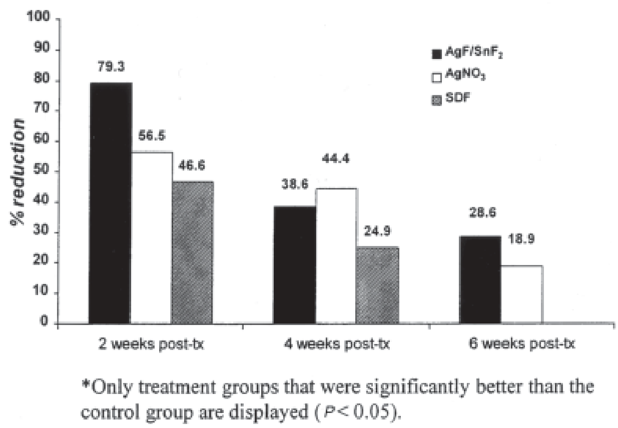

Fig 3. Inhibition of lesion progression in % (Only treatment groups that were significantly better than the control group are displayed [P>0.05]).

(29% and 19% reduction in lesion depth progression, respectively). Lesion depth for all groups increased steadily with time, which indicated no adverse statistical interaction. A typical baseline lesion is displayed in Fig 4.

Daily measurement of the pH in the flasks yielded an average pH of 3.95 (SD 0.3). The day after the application of the treatment agents the readings were as follows: AgF/SnF_2, pH=4.58; CHX, pH=4.32; $AgNO_3$, pH=5.63; SDF, pH=4.68; control group, pH=3.96.

Discussion

The purpose of this study was to compare four different known treatment regimens in their ability to delay carious lesion progression under controlled circumstances. The bacterial caries model used provided a constant challenge by MS and L as well as a constant supply of fermentable carbohydrates to simulate the conditions present in the oral cavity of children at high risk for dental caries. The use of an in vitro caries model allowed for direct, controlled, and accurate comparison of the efficacy of the treatment regimens being investigated.

As treatment agents for this study, AgF/SnF_2 and SDF were chosen because they have been standard regimens in Australia and Japan for treating caries in younger children or children that have only limited access to dental care. $AgNO_3$ was included because it was used in the United States and Europe until the late seventies and because its efficacy has been discussed in such diametric terms. The decision regarding which

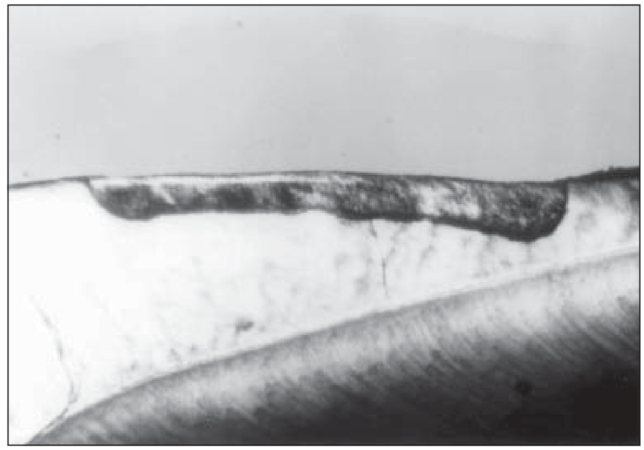
Fig 4. Sample of a typical baseline lesion.

CHX preparation to utilize was more difficult. There were two main groups to choose from: CHX gels and CHX varnishes. CHX gels in various concentrations have been successfully used to reduce MS levels in saliva and plaque. CHX varnishes, also in a variety of concentrations, have been directly applied to teeth in order to eliminate MS from the hard tissues in the oral cavity.[17, 18, 20, 21] The varnish base alone has been proven to be somewhat effective in reducing MS levels,[17] whereas varnishes containing high (40%) concentrations of CHX have been found capable of reducing MS for up to eight weeks after a single application. Because our laboratory model did not incorporate a mechanical abrasive component, inclusion of a sticky varnish may have led to artificially good results for this group, which might not have been true for an in vivo situation where mechanical influences always occur. It was decided, therefore, to use a water soluble CHX gel.

In the literature, a number of methods to assess lesion progression have been reported; A comparison of lesion depth on bitewing radiographs,[11, 22–24] photographs of clinically visible lesions,[25] dmft/DMFT indexes,[15, 26, 27] determination of the effects on the MS counts of the preparations to be tested,[17, 19, 28,29] and a measurement of bacterial growth inhibition properties via standard zone inhibition assay.[30] We assessed the efficacy of the agents by measuring the increase of lesion depth for each time period by photomicroscopy of the sectioned specimens.

Under the constraints of this laboratory model system, a one-time application of a AgF solution followed by a SnF_2 paste was able to slow down caries progression in a six-week period by 29% compared to a control group. The corresponding reduction for $AgNO_3$ was 19%. These numbers cannot be compared directly to those reported by Craig[15] because he presented findings from a clinical study on caries progression in primary molars. On average, he applied the metal fluoride regimen 1.2 times within the two-year study period and reported that only 31% of the observed 281 lesions needed a restoration. In contrast, figures by Murray and Majid[31] demonstrate that in a 12-month period 69 of 71 incipient (in enamel only) carious lesions in deciduous teeth progressed into dentin, and Van Erp and Meyer-Jansen[32] found that 94% of the studied lesions penetrated into dentin in 12 months or less.

A repeated 0.4% SnF_2 gel application[33] was proven to be ineffective to arrest incipient nursing caries in two- to three-year-old children, but the negative result was primarily due to noncompliance by the caretakers involved. The more favorable outcome of SnF_2 in our study could be attributed to the higher concentration (10 versus 0.4%) and the fact that it was used in combination with AgF.

Oppermann and Johannsen[34] compared 5 mM solutions of $AgNO_3$, AgF, and SnF_2 and found that acid production in plaque after a sucrose challenge was suppressed less with SnF_2. Our data indicated that all Ag and F^- containing agents were successful in inhibiting lesion progression. Results contrary to ours regarding the efficacy of $AgNO_3$ and CHX were found by Wåler and Rölla.[35] They described a four-day trial during which the agents were applied twice a day for one minute. In this clinical test, CHX could accumulate in the oral mucosa and be slowly released and thus exhibit its strong antimicrobial potential, whereas $AgNO_3$ was diluted and washed out more rapidly.

This phenomenon could account for why CHX exhibited only limited activity in our study. In an in vitro environment, CHX could not accumulate in soft tissues, but could only act strongly during the subsequent two days following its application until the media were changed again. From that time on, most of the water soluble gel was gone as it obviously dissolved in the first medium. Subsequently, CHX behaved like the control group, as virtually no remaining active agent was available in hard tissues, resulting in similar lesion progression profiles as the control group at all three time periods. The other agents, however, could act much longer against the cariogenic challenge due to the precipitation of metallic silver and the deposition of F^- in enamel.

McDonald's[22] findings that the effect of $AgNO_3$ were identical to that of SnF_2 coincide with our results that AgF/SnF_2 and $AgNO_3$ were statistically not different in their efficacy. Nishino and Massler,[36] in contrast, concluded in their study that $AgNO_3$, when compared to SDF and SnF_2, was not effective at all in reducing mean caries scores in rats' teeth.

In a clinical study, McDonald and Sheihan[23] compared chemical caries arrestment regimens with minimal cavity preparation and found no significant caries reduction when compared to a no treatment control group. The outcomes of simple operative procedures were superior to application of a 10% SnF_2 gel or a SDF/SnF_2 regimen.

At day 56 of this study the lesion depth for teeth treated with SDF was not statistically different from the CHX and the control group, but AgF/SnF_2 and $AgNO_3$ were. The success of these groups can be attributed to highly concentrated silver in the form of AgF or $AgNO_3$ and to the high F^- content of AgF and AgF/SnF_2. Fluoride concentration in a 40% AgF solution based on calculation should be 59,900 ppm. Gotjamanos[37], however, found F^- levels of the AGF Silverfluoride preparation (Creighton Dental Supply, Sydney) to be as high as 100,000 ppm and expressed concern about its use in pediatric dentistry.

Silver is known for its antimicrobial effect as heavy metal and for its formation of a diffusion barrier in plaque by precipitating bacterial proteins. Fluoride assists in remineralization, slows down plaque growth, and exerts antimicrobial activity by inhibiting the production of glucosyltransferase.[38] SnF_2, even in low concentrations, has a growth inhibiting potential on dental plaque. Ostela and Tenovuo[30] found that a AgF/SnF_2 gel with a 1.2% F^- content was more effective in inhibiting MS growth on an agar test plate than a 1% CHX gel.

The daily recording of the pH in the current study yielded very consistent values throughout all 28 reading times, except the two days following the application of the treatment agents, when only in the control group the usual pH value was found. This could be explained by the fact that the supernatant amount of the agents which dissolved from the application site into the medium reduced bacterial growth in these media, resulting in a lower acid production. After the following routine change of the media the pH readings were back to normal. This observation allows for the conclusion that mainly local effects within the tooth structure have contributed to lesion progression inhibition.

Shellis[39] compared the different reactivity of primary and permanent tooth enamel in an acidic caries model, and found that mean lesion depth generated was on average 75% greater in primary teeth due to the higher porosity of primary enamel. It is likely that the effects of this study on permanent teeth would have been more pronounced if primary teeth had been used.

Future tests should include a mechanical component to be able to compare CHX or F^- containing varnishes with the successful agents of this study, the potential of which is now known. Another goal could be to develop varnishes containing higher concentrated silver and fluoride preparations as tested.

The regimens found to be efficacious in this study could potentially be useful in selected clinical situations. In some young children, it may be possible to arrest the clinical progression of ECC sufficiently to allow delaying definitive care until they are old enough to cooperate for conventional treatment. In other patients who are waiting for an oral rehabilitation in the operating room, these agents could possibly create more favorable preconditions for the dental procedures to be performed there, by slowing down or even arresting the carious process as well as hardening the tooth structures involved. Repeated applications may even extend the beneficial effects.

Application of all silver containing agents results in precipitation of black metallic silver, therefore, appropriate precautions must be applied to prevent spilling and staining of adjacent tissues.

Conclusion

The use of chemotherapeutic agents to reduce caries progression shows promise. In this in vitro study, single applications of either AgF/SnF_2 or AgNO proved to be successful methods of reducing carious lesion progression over a six-week period of time. Compared to a control group, reduction in lesion depth progression for these two treatment regimens was 29% and 19%, respectively. Both treatments were significantly better ($P<0.05$) that the other groups, although not significantly different from each other.

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