Alteration in salivary and plaque S. Mutans in adults brushing with 0.4% SnF₂ gel once or twice a day

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Abstract

Two clinical trials were performed to investigate the antibacterial effects of SnF₂ and to explore the apparent selective reduction of salivary S. mutans. In the first clinical trial, subjects were followed through baseline periods interposed between periods of either once or twice daily brushing with 0.4% SnF₂. At weekly intervals, salivary samples from each subject were quantitated for S. mutans and total colony-forming units. SnF₂ reduced salivary S. mutans more than the total CFU, and twice daily brushing was found more effective than brushing once a day. The second trial sampled both plaque and saliva in the same subjects to investigate whether the selectivity of SnF₂ against S. mutans was the result of a site-specific effect. Similar percentage reductions in S. mutans were found in both saliva and plaque, suggesting that the effect of SnF₂ against S. mutans must be due to something other than SnF₂ affecting primarily the bacteria on teeth.

Caries reduction by fluoride traditionally has been ascribed to its physicochemical interaction with enamel. Recent research, however, also has been directed at the effect that different fluoride compounds have on bacterial growth and plaque formation. It appears that one fluoride compound, SnF₂, has greater antimicrobial properties than other commonly used fluoride compounds. The antimicrobial properties of SnF₂ appear to affect S. mutans, the bacterium associated with dental caries, more than other nonpathogenic bacteria in the mouth. This "selective" reduction of S. mutans by SnF₂ first was observed in a study of 22 rampant caries subjects who rinsed twice a day with either NaF or SnF₂. Those subjects rinsing with SnF₂ had significant reduction of salivary S. mutans, while salivary total colony-forming units (CFU) and salivary lactobacilli were not affected by the SnF₂ rinsing. Subsequently, there have been at least 5 reports showing selective reduction of S. mutans by SnF₂ using various concentrations, frequency of use, and delivery systems.

Several theories have been proposed to explain the selectivity of SnF₂ against S. mutans. One explanation is that SnF₂ inhibits acid formation in plaque for several hours, and the increase in plaque pH may create an ecologic disadvantage for S. mutans. Another theory is that tooth surfaces disinfected with an antimicrobial agent such as SnF₂ are recolonized more easily with S. sanquis because of its greater oral reservoir. It also seems possible that the noted selective reduction of salivary S. mutans is an artifact of the sampling procedure. If an antimicrobial agent primarily affected bacteria adhering to teeth, then the number of tooth-associated bacteria shed to the saliva could appear to be selectively reduced compared to the rest of the salivary flora.

This paper reports on 2 clinical trials, using similar methodologies, to examine a delivery system for SnF₂ and to further explore the reported selective reduction of S. mutans by SnF₂. The first clinical trial used a time series approach to explore the effects on bacterial reduction caused by varying the intervals of application of SnF₂. As a result of the frequent recovery periods in this approach, the authors also were able to examine the data for possible carry-over effects of SnF₂ past the treatment intervals, and for possible bacterial adaptations to SnF₂. The second trial sampled both plaque and saliva in the same subjects to determine whether a reduction in the number of sites on the teeth that seed S. mutans into saliva causes the apparent salivary reduction of S. mutans by SnF₂.
Methods and Materials

The subjects of this study consisted of 17 adults, 20-39 years old, having greater than $2 \times 10^8$ S. mutans/ml saliva, selected from 27 employees of the University of Connecticut Health Center who were screened for sufficient salivary S. mutans levels. During the 22 weeks of the study, subjects were sampled weekly to monitor their total CFU and S. mutans levels, while they participated in a time series experiment. A time series experiment follows the same subjects through intervals of baseline periods interposed between a progression of experimental periods.

This specific time series consisted of an initial 2-week baseline period in which subjects gave weekly salivary samples, but no modification of the subject’s oral hygiene habits or dentifrice took place. After this initial baseline period, the subjects were asked to brush their teeth, once daily in the evening for 1 min with a 0.4% SnF$_2$ gel for the next 2 weeks (weeks 3-4). A nonfluoride toothpaste also was given to the subjects for their use ad libitum. Weeks 5, 6, and 7 consisted of a nontherapeutic period in which subjects used only the nonfluoridated toothpaste. On weeks 8-9, the use of the 0.4% SnF$_2$, once a day, was repeated again followed by a 3-week nontherapeutic period. The same experimental approach was used to test twice daily brushing with SnF$_2$. During weeks 13-14 and 18-19, subjects were instructed to brush twice a day with SnF$_2$, while weeks 15, 16, 17, and 20, 21, 22 were interposed nontherapeutic periods where subjects brushed only with nonfluoridated toothpaste.

For the microbiologic sample, subjects provided 1 ml of saliva, stimulated by chewing on a piece of paraffin. Each saliva sample was vortexed, sonicated, serially diluted in 0.05M phosphate buffer (pH 7.0), and 25 µl of each dilution was spread onto thirds of a 10% sheep blood agar plate for estimates of the total aerobic bacteria, and Mitis Salivarius® agar plates containing 0.2 units/ml Bacitracin for estimates of S. mutans. Total CFU were counted with the aid of 20x magnification after 24 hr incubation in a CO$_2$-enriched environment (candle jar) at 37°C. After incubating the MSB agar plates for 96 hr in candle jars, those colonies with morphologic characteristics of S. mutans were counted.

The means of the total CFU and S. mutans for each time interval were calculated and further reduced to total means for treatment periods. Although traditional statistical tests with a time series experiment are questionable due to lack of double blindness, changes of baselines, and potential carry-over effects; paired t-tests still were performed between treatment adjacent nontherapeutic periods to enable more than visual inspection of the data.

Reductions in Plaque Versus Saliva

The subjects of this study were 10 adults, 20-39 years old, who had more than $2 \times 10^8$ S. mutans/ml saliva. Each subject's saliva and plaque were sampled weekly during a 3-week baseline period and a subsequent 3-week experimental period. No modification of the subjects’ oral hygiene habits or dentifrice took place during the baseline period. In the experimental period, the subjects were asked to brush their teeth unsupervised, twice daily for 1 min with the 0.4% SnF$_2$ gel.

The sampling, sonicating, diluting, and plating of saliva for estimation of total CFU and S. mutans/ml saliva were performed as in the previous study. After the saliva samples were acquired from each patient, a pooled dental plaque sample was obtained from each patient by scraping the gingival margins of the teeth with a large dental cleod carrier until the end was covered with plaque (~ 3 mg). The end of the carver containing the plaque then was placed in 4.5 ml of phosphate buffer and the carver was shaken until the bacterial mass was dislodged. The plaque in the buffer then was vigorously sonicated for 20 sec using a sonicator equipped with a microtip. The dispersed bacteria then were diluted further and plated in the same way as the saliva samples. The mean percentage of S. mutans per total flora was calculated for plaque and saliva samples in order to compare the potential reductions in both ecologic niches due to SnF$_2$.

Results

The 17 subjects who volunteered for the study had mean baselines of 9.38 x 10$^7$ total CFU and 3.297 x 10$^8$ S. mutans/ml saliva. Thus, S. mutans accounted for only 0.35% of the subjects’ mean cultivable salivary flora. All subjects who initially started the trial completed the 22-week experimental and baseline periods, and were believed to be cooperative with the use of the agents. No side effects were reported in the study although a few subjects complained about the taste or consistency of both the SnF$_2$ gel and the nontherapeutic toothpaste.

Brushing with SnF$_2$ once a day reduced the salivary S. mutans levels 40% in the first trial period and 59% in the second trial compared to initial baseline levels. When the subjects brushed twice daily with SnF$_2$, the S. mutans levels were 48% lower than the initial base-

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* Gelkam-1030 ppm F; 2950 ppm Sn; Scherer Laboratories: Dallas, TX.

* NASA Dent-Scherer Laboratories: Dallas, TX.
line in both trials. However, comparison of the subjects’ *S. mutans* levels to the adjacent nontherapeutic periods revealed a greater effect of twice daily brushing with SnF<sub>2</sub>. The difference between *S. mutans* counts during the once daily SnF<sub>2</sub> period and the adjacent nontherapeutic periods was 18 and 33%, respectively, for the first and second trial; whereas in the trials that used SnF<sub>2</sub> twice daily, the *S. mutans* reductions in the experimental periods were 51 and 58% compared to adjacent nontherapeutic periods (p < .05). Furthermore, a carry-over effect of the *S. mutans* reduction is suggested by the mean reduction of *S. mutans* levels in the first 2 nontherapeutic periods (weeks 5-7 and 10-12). However, no reduction of *S. mutans* was evident in the last 2 nontherapeutic periods (Fig 1).

Little change in salivary total CFU due to brushing with SnF<sub>2</sub> or the nonfluoride dentifrice was evident. Only in the second experimental period (weeks 8-9) was there a significant reduction in total CFU (27%) compared to baseline. The mean total CFU in the remainder of the trial and baseline periods approximated baseline levels (Fig 2).

**Reduction in Plaque Versus Saliva**

The 10 subjects initially had means from the 3 baseline salivary samples of 8.32 x 10<sup>7</sup> total CFU, and 2.38 x 10<sup>5</sup> *S. mutans*/ml saliva. The percentage of salivary *S. mutans* per salivary total CFU was thus 0.29% prior to treatment (Fig 3). The baseline plaque pooled recoveries were 1.24 x 10<sup>9</sup> total CFU and 4.24 x 10<sup>6</sup> *S. mutans* per sample, yielding a higher ratio (3.42%) of *S. mutans*/total CFU (Fig 4).

**Fig. 1.** Mean levels of salivary *S. mutans* in 17 subjects who brushed either once or twice daily with 0.4% SnF<sub>2</sub>.

**Fig. 2.** Mean levels of salivary total CFU in 17 subjects who had intervals of brushing once or twice daily with 0.4% SnF<sub>2</sub> or with a nonfluoridated dentifrice.

**Fig. 3.** Percentage of *S. mutans* in saliva samples in 10 subjects during a 3-week baseline period and during 3 weeks where they brushed their teeth twice daily with 0.4% SnF<sub>2</sub>.

During the time that the subjects brushed twice daily with SnF<sub>2</sub>, the percentage of *S. mutans* in saliva was found to be 0.16% (65% reduction), while the
logically acceptable that the specific suppression or caries activity.

S. mutans/ml reduction of the oral flora with antiseptics or antimicrobial action of SnF2 against S. mutans is the result of site-specific effects of the agent.

The present finding of a reduction of S. mutans while the total CFU are unaffected upholds the concept of a selective antimicrobial action of SnF2 against S. mutans reported in other studies. The reduction of S. mutans infection in the oral cavity is a treatment goal where they brushed their teeth twice daily with 0.4% SnF2.

percentage of S. mutans in pooled plaque was 1.14% (77% reduction).

Discussion

These 2 clinical trials confirm previous studies showing that SnF2 reduces S. mutans in the oral cavity even for a period after discontinuation of the topical treatment. These trials also extend the understanding of some other variables such as: the frequency necessary to optimize the S. mutans reduction; effects of the agent in a low-risk population; bacterial adaptation to the agent; and whether the noted reduction of S. mutans is the result of site-specific effects of the agent.

The authors initially hypothesized that a large decrease of S. mutans in dental plaque — due to SnF2 primarily affecting the tooth site — might be reflected in the saliva as a specific reduction of S. mutans. The data, in general, do not support this as a reason for the specific S. mutans reduction in the saliva. The authors currently are conducting in vitro studies examining the effect of SnF2 on isolates of various oral bacteria to explore further the mechanism of the selective effect of SnF2 on S. mutans.

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