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Alteration in salivary and plaque *S. Mutans* in adults brushing with 0.4% SnF_2 gel once or twice a day

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Abstract

Two clinical trials were performed to investigate the antibacterial effects of SnF_2 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% SnF_2. At weekly intervals, salivary samples from each subject were quantitated for* S. mutans *and total colony-forming units. SnF_2 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 SnF_2 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 SnF_2 against* S. mutans *must be due to something other than SnF_2 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, SnF_2, has greater antimicrobial properties than other commonly used fluoride compounds.[1] The antimicrobial properties of SnF_2 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 SnF_2 first was observed in a study of 22 rampant caries subjects who rinsed twice a day with either NaF or SnF_2. Those subjects rinsing with SnF_2 had significant reduction of salivary *S. mutans*, while salivary total colony-forming units (CFU) and salivary lactobacilli were not affected by the SnF_2 rinsing.[2] Subsequently, there have been at least 5 reports showing selective reduction of *S. mutans* by SnF_2 using various concentrations, frequency of use, and delivery systems.[3-7]

Several theories have been proposed to explain the selectivity of SnF_2 against *S. mutans*. One explanation is that SnF_2 inhibits acid formation in plaque for several hours, and the increase in plaque pH may create an ecologic disadvantage for *S. mutans*.[3] Another theory is that tooth surfaces disinfected with an antimicrobial agent such as SnF_2 are recolonized more easily with *S. sanquis* because of its greater oral reservoir.[4] 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 SnF_2 and to further explore the reported selective reduction of *S. mutans* by SnF_2. The first clinical trial used a time series approach to explore the effects on bacterial reduction caused by varying the intervals of application of SnF_2. 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 SnF_2 past the treatment intervals, and for possible bacterial adapatations to SnF_2. 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 SnF_2.

Methods and Materials

The subjects of this study consisted of 17 adults, 20-39 years old, having greater than 2 x 10^5 *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.[8] 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[a] for the next 2 weeks (weeks 3-4). A nonfluoride toothpaste[b] 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*.[9] 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 x 10^4 *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 cleoid carver 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 10^5 *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-

[a] Gelkam-1030 ppm F^-, 2950 ppm Sn^{++}; Scherer Laboratories: Dallas, TX.

[b] NASAdent-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_2. The difference between *S. mutans* counts during the once daily SnF_2 period and the adjacent nontherapeutic periods was 18 and 33%, respectively, for the first and second trial; whereas in the trials that used SnF_2 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_2 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 8.32×10^7 total CFU, and 2.38×10^5 *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 1.24×10^8 total CFU and 4.24×10^6 *S. mutans* per sample, yielding a higher ratio (3.42%) of *S. mutans*/total CFU (Fig 4).

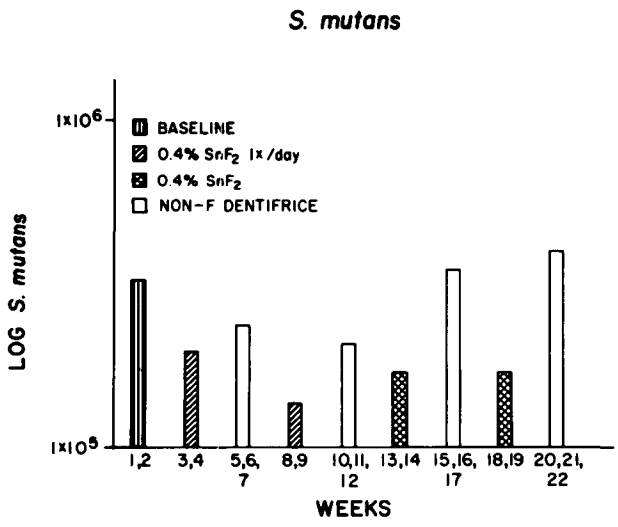

FIG 1. Mean levels of salivary *S. mutans* in 17 subjects who brushed either once or twice daily with 0.4% SnF_2. Two week-long experimental periods were separated by 3 week-long recovery periods where subjects brushed with a nonfluoridated dentifrice.

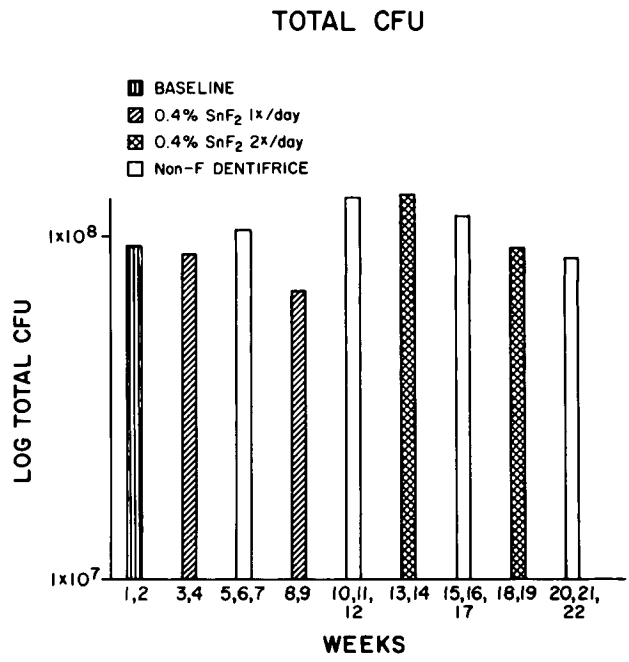

FIG 2. Mean levels of salivary total CFU in 17 subjects who had intervals of brushing once or twice daily with 0.4% SnF_2, or with a nonfluoridated dentifrice.

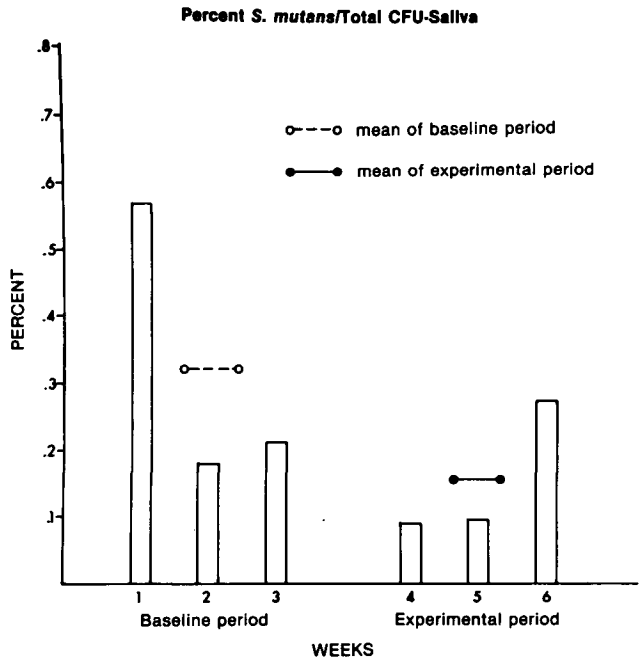

FIG 3. Percentage of *S. mutans* in saliva samples in 10 subjects during a 3-week baseline period and during 3 weeks where they brushed their teeth twice daily with 0.4% SnF_2.

During the time that the subjects brushed twice daily with SnF_2, the percentage of *S. mutans* in saliva was found to be 0.16% (65% reduction), while the

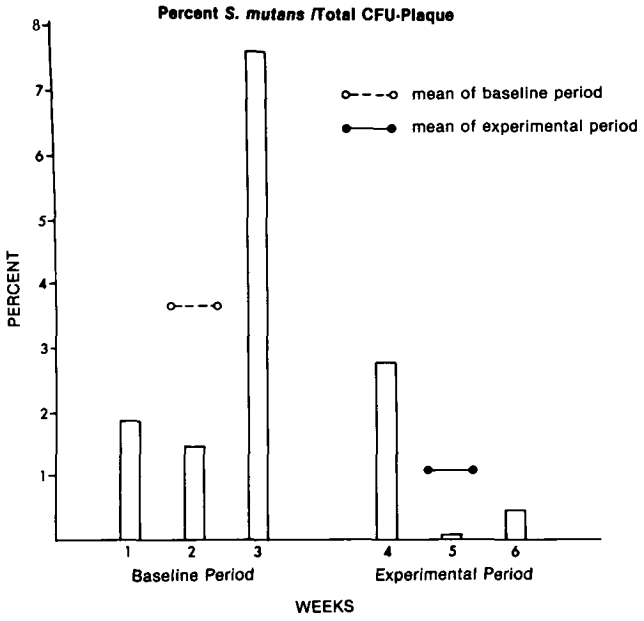

FIG 4. Percentage of *S. mutans* in pooled plaque samples in 10 subjects during a 3-week baseline period and during 3 weeks where they brushed their teeth twice daily with 0.4% SnF_2.

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

Discussion

These 2 clinical trials confirm previous studies showing that SnF_2 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 present finding of a reduction of *S. mutans* while the total CFU are unaffected upholds the concept of a selective antimicrobial action of SnF_2 against *S. mutans* reported in other studies.[2-7] The reduction of *S. mutans* infection in the oral cavity is a treatment goal of most antimicrobial therapy since this organism has been shown to be associated strongly with caries in humans and animals.[10] Furthermore, high levels of *S. mutans* in the mouth are correlated with increased caries increment;[11,12] subjects with more than 200,000 *S. mutans*/ml saliva have been shown to be at risk for development of new carious lesions.[13] Nonspecific reduction of the oral flora with antiseptics or antibiotics also has been shown to affect *S. mutans* levels and caries activity.[13-15] However, it seems more biologically acceptable that the specific suppression or elimination of *S. mutans* without reducing nonpathogenic bacteria, as found with SnF_2 treatment, would be superior to a nonspecific chemotherapeutic approach in preventing dental caries.

The carry-over effect of SnF_2 on the reduction of *S. mutans*, noted in the second and third baseline periods of the long-term study, also confirms other studies that have shown that *S. mutans* levels are reduced in subjects several weeks after the discontinuation of the SnF_2 topical treatment.[7,16] Of interest in this study is the finding that in the fourth and fifth recovery periods, no carry-over effect was evident. Bacterial adaptation to SnF_2 is suggested from these data; however, the lack of a control group and the high variability of bacterial recoveries makes the finding inconclusive. Bacterial adaptation to SnF_2 also is suggested in 2 clinical trials that have exceeded 1 year.[17,18] Further studies are necessary to examine possible diminution of effects with long-term SnF_2 use.

One of the purposes of the time series study was to evaluate the difference between using 0.4% SnF_2 once or twice a day. Twice daily brushing with the SnF_2 gel clearly had a greater effect on *S. mutans*. Other oral antimicrobial agents also require twice daily usage for effectiveness.[19] The average reductions of *S. mutans* in the present studies (18-59%), however, were not as large as that noted in other studies in which subjects were selected by high caries risk or high *S. mutans* levels.[5-7,16] The subjects in the current studies, employees of a health center, had lower baseline levels of *S. mutans* than other populations previously tested. Perhaps the greatest benefit of the antimicrobial therapy with SnF_2 will be realized in subjects having high caries activity associated with elevated *S. mutans* levels.

We also found that the reduction of *S. mutans* due to SnF_2 in the pooled plaque sample was similar to that found in the saliva. Comparable results have been reported by Svanberg and Rölla,[3] who found that 0.2% SnF_2 mouthrinse produced a tenfold reduction of *S. mutans* in both proximal plaque, fissure plaque, and in saliva. However, their study found a hundredfold reduction of *S. mutans* on the buccal surfaces.

The authors initially hypothesized that a large decrease of *S. mutans* in dental plaque — due to SnF_2 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 SnF_2 on isolates of various oral bacteria to explore further the mechanism of the selective effect of SnF_2 on *S. mutans*.

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