High strontium addition to chlorhexidine–fluoride gel does not increase its caries-preventive effect in rats

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One hundred Osborne–Mendel rats were weaned at the age of 21 to 22 days, inoculated with *Streptococcus mutans* in the mouth, and fed a semisynthetic diet for the next 43 days. The control group received no treatment. The study groups received gel applications on their molars with placebo, chlorhexidine–fluoride (CXF), CXF plus 50 ppm Sr, or CXF plus 250 ppm Sr daily for the first 21 days of the experiment. Although caries was significantly reduced by CXF and CXF plus 50 ppm Sr treatments, the Sr additive did not significantly improve the caries-preventive effect of CXF. The addition of 250 ppm Sr to the CXF gel seemed markedly to weaken the effect of CXF. □*Antimicrobial agents; caries; laboratory animals*

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It has been shown that chlorhexidine and fluoride have an additive or even a synergistic depressing action against mutans streptococci (1–3) and also an anticaries effect better than that of fluoride alone in man (4, 5); for a review see Luoma (6). There is also evidence that strontium and fluoride have a synergistic effect in reducing the acid reactivity of apatites (7, 8). The possible additive or synergistic antimicrobial action of fluoride and strontium against cariogenic microbes has not been established, perhaps because the stronger effect of fluoride easily masks the effect of strontium on, for example, intracellular polysaccharide accumulation (9). For other details, see the review by Curzon (10).

More recent in vitro and animal experiments have given some promising results (11, 12) for strontium as a beneficial component in fluoride or chlorhexidine–fluoride applications, but there are also results that show no benefit of strontium addition (13).

In a clinical trial lasting up to 2 years and 9 months, chlorhexidine–fluoride–strontium solution applied twice a day for 5-day periods during the first 2 years, with 2-week intervals of no treatment, was ineffective in preventing caries (14). In the same trial chlorhexidine–fluoride solution applied with the same protocol throughout the trial period of 2 years and 9 months produced a trend of 35% reduction in caries increment, but this reduction was not statistically significant, partly due to the small overall caries increments. Yet, it seemed to be in line with the significant reduction obtained previously with the same solution (4). Furthermore, when the strontium concentration in the chlorhexidine–fluoride–strontium solution was reduced from the original 500 ppm to 15 ppm for the last 9 months of the trial, caries increment during this short period was lower than in the chlorhexidine–fluoride group. Again, however, the difference was not significant.

In a recent in vitro caries model study (15) the addition of strontium to the chlorhexidine–fluoride gel improved significantly its effect in preventing enamel softening during sucrose fermentation of *Streptococcus sobrinus* 'plaque'. The concentration of 250 ppm strontium had a significantly better additive effect than the concentration of 15 ppm.

Since the optimum concentration of strontium to be applied with chlorhexidine and fluoride has not been clearly established, the aim of the present study was to compare the efficacy of 50 and 250 ppm strontium in chlorhexidine–fluoride gel in preventing caries in rats.

Materials and methods

The ethical committee for animal experimentation of the University of Kuopio approved the following study plan: a total of 100 Osborne–Mendel rats with normal rat flora were weaned when 21 to 22 days old and divided randomly into 5 groups. On the day of weaning their mouths were inoculated once with 0.1 ml fresh 18-h thioglycollate culture of *Streptococcus mutans* Ingbritt, after which they were kept in stainless steel mesh-bottomed cages, three to four rats per cage. The powdered food mixture Diet 2000 with 56% sucrose (16) and distilled water were available ad libitum for 43 days. To monitor the presence of *Strep. mutans* in the oral cavity, the teeth of the rats were wiped once a week throughout the experiment with cotton swabs, and cultures were made on mitis salivarius bacitracin (MSB) agar, which is selective for *Strep. mutans*. After anaerobic incubation for 48 h the total number of colony-forming

Table 1. Initial weight and weight gains of rats (g) during the 6-week study period (mean ± SD)

Group	No.	Initial weight	Weight gain
C (control)	20	34.9 ± 6.1	147.7 ± 29.0
P (placebo)	20	33.8 ± 4.7	148.1 ± 25.2
CXF	20	35.3 ± 5.7	148.7 ± 28.0
CXFS-50	20	35.1 ± 5.4	137.8 ± 24.3
CXFS-250	20	34.9 ± 5.0	152.2 ± 29.5

units (CFU) were calculated from the plates, using a binocular microscope.

Since one of our goals was to find further indications for periodic application of chlorhexidine–fluoride combinations with additional components, instead of continuous applications, a protocol of applications for 21 days, followed by a 21-day-period without treatment was adopted. Accordingly, starting on the day after inoculation the groups received a daily application of the following gels for the next 21 days:

Group C: Control, no treatment.

Group P: Placebo gel (pH 5.8) consisting of 0.017 M succinic acid, 0.029 M NaOH, 2.0% methylhydroxypropyl cellulose (4000 Serva, Feinbiochemica GmbH & Co., Heidelberg, Germany), and 0.01% menthol.

Group CXF: The above gel with 0.20% chlorhexidine gluconate (CHX) and 0.16% sodium fluoride (NaF).

Group CXFS-50: The above gel with 0.20% CHX, 0.16%NaF, and 50 ppm strontium (Sr), as $SrCl_2$.

Group CXFS-250: The above gel with 0.20% CHX, 0.16% NaF, and 250 ppm Sr, as $SrCl_2$.

An amount of 100 µl of gel was applied once a day on the molars of the rats with a piece of rubber sponge fixed in small forceps. After the experimental period postweaning weight gains were determined, and the animals were guillotined. The jaws were removed, cleaned free of the soft tissues and plaque, and preserved in ethanol. The lower jaws and the left half of the upper jaw were hemisectioned sagittally with a thin steel saw and used for caries scoring. Smooth-surface caries was diagnosed with the method of Keyes (17). Then the sections were stained with Schiff's reagent, and caries in occlusal fissures and on approximal surfaces was scored in four grades (A, T, B, C) and cumulated in accordance with the Zürich method (18), without the investigator knowing which group the animal belonged to.

Statistical methods

The comparisons between groups were made using analysis of variance (ANOVA). Scheffé's multiple-range test at a 5% level of significance was applied for pairwise comparison.

Results

During the study period one rat died and was replaced. Analysis of variance showed no significant differences between the groups in the mean initial weights or in the mean weight gains (Table 1). The mean numbers of *Strep. mutans* after 3 weeks' gel treatment in the control, placebo, CXF, CXFS-250, and CXF-50 groups were 2.3×10^3, 8.0×10^3, 0.8×10^3, 1.8×10^3, and 0.3×10^3, respectively. After 6 weeks (at the end of the study period) the respective numbers were 2.1×10^3, 1.3×10^3, 1.3×10^3, 3.0×10^3, and 3.3×10^3. There were a few rats in every group in whose samples *Strep. mutans* were not detected after inoculation.

Because only two enamel smooth-surface lesions were found, these were omitted from the analyses. Approximal caries lesions were found only in two groups. In the placebo group the mean number of approximal A lesions was 0.4 and B lesions 0.1, and in the CXF group the mean number of A lesions was 0.1. Fissure caries (Fig. 1) was significantly reduced by CXFS-50 gel treatment compared with placebo gel treatment and the controls (A, T, and B lesions). Since it is difficult to differentiate between delayed maturation and the A lesions, the values of T and B lesions are decisive. The difference in mean caries scores between the CXFS-250 and the CXFS-50 group was also significant at 0.05% level in T lesions. Placebo gel treatment itself seemed to promote fissure caries, and the promotion was statistically significant at the 0.05% level in T lesions compared with controls. CXF gel treatment reduced caries more than the same gel with the addition of

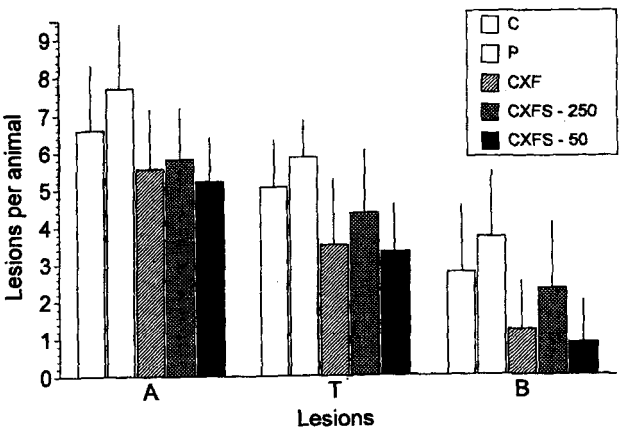

Fig. 1. Mean number of fissure caries lesions in rats fed for 43 days. A = initial enamel lesions; T = lesions extending to the dentin–enamel junction; B = small-dentinal lesions. Vertical bars represent standard deviations.

250 ppm strontium, but the difference was not statistically significant.

Discussion

Although the numbers of *Strep. mutans* in different groups and at different time points are not comparable per se owing to non-standardized sample size, the results indicate that most of the rats harbored *Strep. mutans* after inoculation and that the antimicrobial treatment did not eliminate them completely. Thus *Strep. mutans* most likely contributed to the caries increment found in this study. The occurrence of buccolingual and approximal caries was lower than expected even in the two control groups. One explanation for this might be that the sucrose we used was not very fine (80% of the particles remained on a 60-mesh screen, whereas all passed the 40-mesh screen). This may have reduced plaque accumulation and thereby also caries initiation on those surfaces. For the same reason, the occurrence of fissure caries could be lower than generally in rats kept on this formula. The tendency of the placebo gel to promote caries may simply be due to the slight acidity of the gel (pH 5.8), which may have slightly favored the aciduric *Strep. mutans* used in the study.

The gel with 50 ppm strontium was only slightly more effective than the CXF gel in reducing caries, and the difference was not statistically significant. It is noteworthy that the caries reduction by the former was of the same magnitude as that obtained in rats by daily topical application of chlorhexidine–fluoride solution with 1000 ppm strontium for 48 days (12).

The finding that addition of 250 ppm strontium seemed to reduce the efficacy of the chlorhexidine–fluoride gel is very important but unexpected in the light of our previous in vitro study (15) in which addition of strontium to the chlorhexidine–fluoride gel improved the combination in preventing enamel softening and pH fall during bacterial fermentation. There was a tendency towards greater protection in relation to increased strontium concentration, the concentration of 250 ppm giving the best protection. It was also shown in that study that the incubation period of 24 h did not alter the structure of the enamel surface. However, with longer incubation periods (11) the strontium content of the enamel surface and at a depth of 100 μm was notably higher in samples with strontium plus fluoride treatment than in samples with strontium or fluoride treatment alone. Thus in caries models the time factor, involving, for example, the accumulation of elements in enamel and plaque, is worth considering in our further work.

The highly different caries-preventive potential of CXF gel with 50 or 250 ppm Sr in the present study, together with our previous results on the effect of strontium on caries, suggests that, for caries prevention, there is an 'optimum' concentration range for Sr in the plaque–enamel milieu. This may vary with the model, vehicle, or species involved. An Sr content of about 6 ppm in drinking water (having an F content between 1.0 and 1.2 ppm) was associated with the lowest caries prevalence in man (19) compared with lower or higher concentrations of Sr. Further experiments are still needed, however, to justify that idea of an optimum concentration range or ranges of strontium to be used in association with chlorhexidine and fluoride. Frankly, however, the idea of improving the anticaries effect of the CXF combination through Sr additions was not supported to any significant extent in the present study. The prime reason for this may be the generally weaker protective potential of Sr, compared with, for example, that of fluoride.

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