Antimicrobial effect of fluoride mouthrinse on mutans streptococci and lactobacilli in saliva

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Abstract

Purpose: This study was designed to determine whether the long-term use of fluoride mouthrinse affects the salivary levels of mutans streptococci and lactobacilli.

Methods: The subjects consisted of 414 school children aged 7, 10, and 12 yrs. Among these subjects, 243 children had received fluoride mouthrinse since 5 years of age at nursery schools, and comprised the "mouthrinse group." The remaining 171 children, the "no mouthrinse group," had not received fluoride mouthrinse. All of the children received routine dental health education. The levels of mutans streptococci and lactobacilli for the subjects in both groups were measured using Dentocult SM Strip mutans® and Dentocult LB Dip Slide®, respectively. Dental examinations were conducted to obtain data on caries prevalence (dfs and DMFS). Logistic regression analysis was used to control confounding factors: age, dfs, DMFS, frequency of toothbrushing, sum of permanent tooth surfaces sealed, frequency of sweet snacks, frequency of sweet drinks, and the use of fluoridated toothpaste.

Results: There was a significant difference in mean DMFS between the mouthrinse group and no mouthrinse group at all ages. Children with fluoride mouthrinse had lower levels of mutans streptococci, and there was a significant association between the experience of fluoride mouthrinse and the score of Dentocult SM strip® Odds ratio: 0.64, P<0.05. However, there was no significant relation between the experience of fluoride mouthrinse and the score of Dentocult LB Dip Slide®. The results of this study demonstrated that the long-term use of fluoride mouthrinse affected the detectable levels of mutans streptococci, but did not affect the levels of lactobacilli.

Conclusions: These results suggest that fluoride mouthrinse might contribute to reducing the number of mutans streptococci. (Pediatr Dent 23:113-117, 2001)

Since mutans streptococci and lactobacilli have been implicated in the initiation and progression of dental caries, these organisms have been associated with caries risk assessment.[1]

For caries prevention, some antimicrobial agents have been used. For example, chlorhexidine has shown outstanding antimicrobial properties. A significant caries reduction in schoolchildren has been obtained with inhibition of mutans streptococci by chlorhexidine applications.[2] In addition, it is well known that the children participating in fluoride mouthrinse programs have a lower incidence of dental caries.[3-5] Much of the research on the effects of fluoride mouthrinse has been focused on the interaction between fluoride and the dental hard tissues, while little or no attention has been paid to the effects of fluoride mouthrinse on the bacteria in the saliva.

The antimicrobial action of fluoride has also been demonstrated. In an *in vitro* study, a mixed culture with 19 ppm fluoride inhibited the growth of mutans streptococci.[6] A high fluoride concentration in the oral cavity might inhibit acid production by bacteria and may reduce the numbers of certain species of bacteria.[7] Some researchers have also demonstrated lower levels of mutans streptococci in plaque adjacent to fluoride-releasing glass ionomers.[8,9] Furthermore, children using application of 0.5% hydrofluoride solution showed lower levels of mutans streptococci.[10] On the other hand, no different levels of mutans streptococci or lactobacilli were found between subjects using or not using different fluoridated toothpaste.[11]

There were no differences in distribution of children with mutans streptococci between water-fluoridated and non-fluoridated areas.[12] However, the degree of growth inhibition of mutans streptococci or lactobacilli *in vivo* by fluoride mouthrinse remains unknown. It is important to evaluate the actual extent of the antimicrobial effect of fluoride mouthrinse, and a case *in vivo* should be made for estimating its role in caries prevention.

This study was designed to investigate whether the long-term use of fluoride mouthrinse affects the salivary levels of mutans streptococci or lactobacilli.

Methods

The subjects consisted of 414 children aged 7, 10, and 12 years, taking different stages of dentition into account. They attended three neighboring schools in two areas. The schoolchildren lived in a similar social and economic environment. Each area was a sightseeing resort which had a ground hot spring. Furthermore, the occupation distribution (agriculture/fishing, factory, service) of the parents were the same in both areas. All schoolchildren were Japanese.

Among the subjects, 243 children (mouthrinse group) had received fluoride mouthrinse for 2-7 years; 7-year-old children had received fluoride mouthrinse with 500 ppm sodium fluoride solution daily since they were 5. In addition, the

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Table 1. Mean Number of DMF and df Surfaces in Mouthrinse (M) and No Mouthrinse (No-M) Groups

Age	Group (yrs)	No. of subjects	DMFS (SD)	dfs (SD)
7	M	82	0.02 (0.02)¨	10.67 (1.19)¨
	No-M	51	0.39 (0.16)¨	18.39 (1.82)¨
10	M	72	0.28 (0.08)¨	8.96 (0.94)˙
	No-M	60	2.93 (0.45)¨	12.28 (1.40)˙
12	M	89	0.28 (0.08)¨	2.31 (0.37)ns
	No-M	60	4.07 (0.56)¨	2.02 (0.48)ns

ns-not significant, ˙$P<0.05$, ¨$P<0.01$ by Mann-Whitney *U* test.

10-year-old children had received fluoride mouthrinse with 500 ppm sodium fluoride solution daily for 2 years (from age 5 to 6), and received fluoride mouthrinse with 2000 ppm sodium fluoride solution weekly for 3 years (from age 7 to 9). The 12-year-old children had received fluoride mouthrinse with 500 ppm sodium fluoride solution daily for 2 years (from age 5 to 6), and received fluoride mouthrinse with 2000 ppm sodium fluoride solution weekly for 5 years (from age 7 to 11). The fluoride mouthrinse was performed after lunch at the nursery schools and at about 10 a.m. at the elementary school under the supervision of school teachers. In contrast, 171 children (no mouthrinse group) at two elementary schools had not received fluoride mouthrinse.

In both groups, in addition to regular dental health education once a year from dental hygienists, the children received routine dental health education from the school nurse, teachers, and occasionally the school dentist, including toothbrushing instructions and advice to restrict sweets. Furthermore, non-fluoride-containing pits and fissure sealing with a light-cured resin based material was applied to some teeth.

The children in both groups resided in fluoride-deficient communities (<0.2 ppm fluoride).

Clinical examinations using dental mirrors and explorers, without bite-wing radiographs, were conducted for both groups in the same month to obtain data on caries prevalence (dfs and DMFS) according to WHO criteria by trained and calibrated examiners. The inter- and intra-examiner reliability were 0.95-0.97 and 0.88-0.93 using Cohen's Kappa, respectively,[13] and 96% of the children received dental examinations.

For measurement of the level of mutans streptococci, a Dentocult SM Strip® (Orion Diagnostica, Finland) was used. After 3 min. paraffin chewing, the strips were rotated approximately 10 times on the children's tongues and withdrawn through closed lips to remove excess saliva. The strips were then transferred to a selective broth in tubes and incubated for 48 h at 37°C. To measure the level of lactobacilli, the Dentocult LB

Table 2. Comparison of Descriptive Variables of Children with High or Low Salivary Level

		Level of mutans streptococci			Level of lactobacilli		
Variable	Value††	High(≥10^5 CFU/ml)	Low(<10^5 CFU/ml)	*P*<	High(≥10^5 CFU/ml)	Low(<10^5 CFU/ml)	*P*<
No. of subjects		174	240		68	346	
Age (SD)		9.34 (0.16)	9.48 (0.14)	ns¨	8.88 (0.25)	9.53 (0.11)	0.05¨
Sweet snacks (N)							
0/day	0	10	18		3	25	
1/day	1	90	150		37	203	
2/day	2	62	57		24	95	
3/day	3	8	9		2	15	
≥4/day	4	1	1	0.05˙	0	2	ns˙
Sweet drinks (N)							
0/day	0	57	86		18	125	
1/day	1	51	75		25	101	
2/day	2	40	56		20	76	
3/day	3	14	12		2	24	
≥4/day	4	4	2	ns˙	0	6	ns˙
Fluoridated toothpaste (N)							
use	1	122	172		51	243	
no use	0	50	64	ns˙	17	97	ns˙
Toothbrushing (N)							
≥2/day	1	95	148		38	205	
<2/day	0	77	89	ns˙	30	136	ns˙
dfs (SD)		10.90 (0.84)	6.77 (0.59)	0.01¨	14.16 (1.47)	7.40 (0.51)	0.01¨
DMFS (SD)		1.37 (0.22)	1.03 (0.61)	ns¨	1.41 (0.40)	1.13 (0.14)	ns¨
Sealant (SD)†		0.50 (0.07)	0.76 (0.08)	0.05¨	0.35 (0.10)	0.71 (0.06)	0.05¨

ns-not significant, ˙ by $\chi 2$-test, ¨ by Mann-Whitney *U* test, †mean number of permanent tooth surfaces sealed.
†† value used in logistic multiple regression analysis.

Table 3. Logistic Multiple Regression and Associated *P*-values

	Dependent variable							
Independent variable	Level of mutans streptococci Odds ratio	Std. Err.	(95%CI)	*P*<	Level of lactobacilli Odds ratio	Std.Err.	(95%CI)	*P*<
Age	-	-	-	-	1.02	0.08	(0.88 - 1.08)	ns
Sweet snacks	1.29	0.20	(0.96 - 1.75)	0.05	-	-	-	-
dfs	1.04	0.01	(1.01 - 1.06)	0.01	1.05	0.01	(1.03 - 1.08)	0.01
FMR·	0.64	0.14	(0.42 - 0.98)	0.05	1.09	0.32	(0.61 - 1.96)	ns
Sealant··	0.87	0.09	(0.72 - 1.06)	ns	0.73	0.12	(0.52 - 1.02)	ns
Prob > chi2	0.00				0.00			
Pseudo R2	0.05				0.07			

·Fluriode mouthrinse, ··Sum of permanent tooth surfaces sealed, ns: not significant.

Dip Slide® (Orion Diagnostica, Finland) was used. Stimulated saliva, produced by 3 min. chewing of paraffin wax, was collected from each subject. Each saliva sample was spotted on duplicate surfaces of an agar plate. This plate was incubated for 4 days at 37°C. Two dentists, different from those who took the measurements, diagnosed the scores for each of these two caries activity tests. The colony density was estimated by the naked eye under sufficient artificial light, and the density was expressed as a score of 0, 1, 2, or 3, according to the manufacturer's instructions, reflecting the amount of colony-forming units per ml of saliva (CFU/ml) on the strip or dip slide.

The percentage of inter-examiner agreement was 93%, and 96% of the children received salivary tests. For Dentocult SM trip mutans®, a score of 0 indicates the complete absence of mutans streptococci or presence in very low numbers; 3 indicates a very dense growth on the strip. Scores 0 and 1 correspond to less than 10^5 CFU/ml saliva. Score 3 corresponds to greater than 10.[6] Score 2 corresponds to a count between score 1 and score 3. Therefore, we used the combined scores for the statistical evaluation, where those 4 scores were grouped into 2 categories[1] (low level ($<10^5$ CFU/ml: scores = 0 and 1, high level; ($\geq 10^5$ CFU/ml: scores = 2 and 3). For the Dentocult LB Dip Slide®, scores 0, 1, 2, and 3 correspond to $10,^3$ $10,^4$ $10,^5$ $10,^6$ respectively.

We also used the same combined scores (low level ($<10^5$ CFU/ml): scores=0 and 1, high level ($\geq 10^5$ CFU/ml: scores = 2 and 3) for the statistical evaluation (Table 2).

Furthermore, the information on the frequency of ingesting sweet snacks and drinks, frequency of toothbrushing, and use of fluoridated toothpaste was obtained by questionnaires distributed by the school teachers and completed by the parents. The questionnaires were completed 1 week before saliva and dental examinations were conducted; 92% of the parents surveyed returned the questionnaires. The procedures, as well as possible benefits, were explained fully to the children's parents, and their informed consent was obtained prior to this study.

The percentage distribution of the different levels of mutans streptococci and lactobacilli, and the caries experience of children were compared between the mouthrinse and the no mouthrinse group. The statistical difference between the groups was tested by χ^2-test or Mann-Whitney *U* test when applicable.

For evaluation of microbiological effect of fluoride mouthrinse, logistic multiple regression analysis was performed. As dependent variables, the levels of mutans streptococci or lactobacilli were used as combined scores. As independent variables, DMFS, dfs, sum of permanent tooth surfaces sealed, toothbrusing frequency, frequency of sweet snacks, frequency of sweet drinks, use of fluoridated toothpaste, experience of fluoride mouthrinse, and age were used (Table 2). For making the final model to evaluate the influence of fluoride mouthrinse on the level of each bacteria, the independent variables, which had *P*-values less than 0.05 according to the statistical difference by χ^2-test or Mann-Whitney *U* test for each variable, were selected.

Results

Table 1 shows caries experience in both the mouthrinse and no mouthrinse groups. There was a significant difference in mean DMFS between the two groups at all ages (Mann-Whitney *U* test, $P<0.01$). Furthermore, a significant difference in mean dfs was demonstrated between the two groups in 7-yr-olds (Mann-Whitney *U* test, $P<0.01$) and 10-yr-olds (Mann-Whitney *U* test, $P<0.05$). The rates of treated teeth among permanent teeth with primary teeth combined were 75

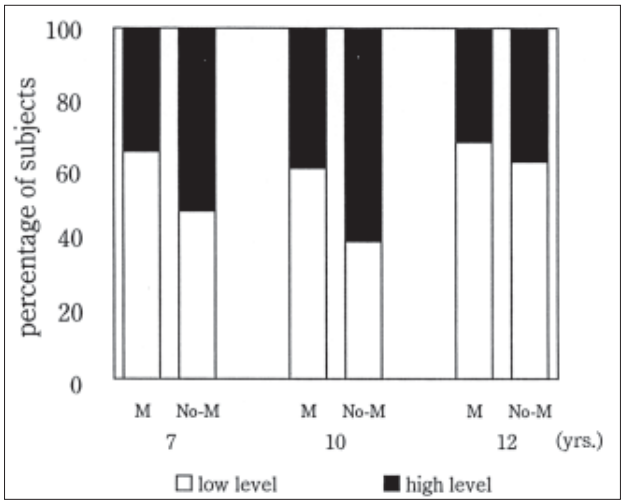

Fig 1. Quantitative distribution of salivary level of mutans streptococci in mouthrinse (M) and no mouthrinse (No-M) groups. Significant differences in the distribution were obtained in 7-yr-olds (χ^2-test, $P<0.05$) and 10-yr olds (χ^2-test, $P<0.05$).

Table 4. Mean Number of Permanent Tooth Surfaces Sealed by High and Low Level of Mutans Streptococci (SM) or Lactobacilli (LB) in Mouthrinse (M) and No Mouthrinse (No-M) Groups

		Mutans streptococci		Lactobacilli	
Age (yrs)	Salivary level of SM or LB	M group sealant¨ (SD)	No-M group sealant (SD)	M group sealant (SD)	No-M group sealant (SD)
7	High	0.00 (0.00)^{ns}	0.22 (0.85)^{ns}	0.00 (0.00)^{ns}	0.50 (1.24)^{ns}
	Low	0.17 (0.70)^{ns}	0.25 (0.90)^{ns}	0.13 (0.63)^{ns}	0.15 (0.71)^{ns}
10	High	0.90 (0.94)^{ns}	0.27 (0.73)^{ns}	0.87 (1.06)^{ns}	0.00 (0.00)$^{\cdot}$
	Low	1.07 (1.26)^{ns}	0.57 (1.16)^{ns}	1.04 (1.16)^{ns}	0.51 (1.04)$^{\cdot}$
12	High	1.31 (1.23)^{ns}	0.30 (0.70)^{ns}	0.67 (0.52)^{ns}	0.20 (0.45)^{ns}
	Low	1.62 (1.35)^{ns}	0.32 (1.18)^{ns}	1.58 (1.34)^{ns}	0.33 (1.06)^{ns}

ns-not significant , $^{\cdot}$ $P<0.05$ by Mann-Whitney *U* test.
¨Mean number of permanent tooth surfaces sealed.

(SD=33)% in the mouthrinse group and 82 (SD=25)% in the no mouthrinse group (data not shown in the table). There was not a significant difference between the two groups (Mann-Whitney *U* test, $P>0.05$).

The percentage distribution of children with each level of mutans streptococci is presented in Figure 1. The mouthrinse group had a higher percentage of children with a low level than the no mouthrinse group for all age groups. Especially in both 7-yr and 10-yr-olds, the differences of proportion occupied by low levels were significant between the two groups (χ^2-test, $P<0.05$). However, there were no significant differences in the percentages of children with a low level of lactobacilli between the two groups. Furthermore, there were no significant differences in mean DMFS between the children with high and low levels of mutans streptococci or lactobacilli at any age in both groups.

Table 2 shows the comparison of the descriptive variables of children with high or low salivary level. The children with a low level of mutans streptococci or lactobacilli had a significantly higher rate of sealant on permanent teeth and less dfs compared to the children with a high level of mutans streptococci. In addition, there was a significant difference in distribution of frequency of sweet snacks between high and low levels of mutans streptococci. The children with a low level of lactobacilli were significantly older than those with a high level.

According to these results, three variables (frequency of sweet snacks, dfs, and number of sealants) for the level of mutans streptococci and three variables (age, dfs and number of sealants) for the level of lactobacilli were selected for the independent variables of the final model (Table 2). The result of logistic multiple regression analysis by the final model is presented in Table 3. The experience of fluoride mouthrinse was associated with the level of mutans streptococci (odds ratio: 0.64, $P<0.05$), after controlling for the frequency of sweet snacks, dfs, and number of sealants. However, the experience of fluoride mouthrinse was not independently associated with the level of lactobacilli.

Discussion

Numerous *in vitro* and animal studies have shown that fluoride has an effect on the carbohydrate metabolism by mutans streptococci.[15] However, there is little evidence that fluoride concentrations as low as those in fluoride mouthrinse could affect the level or cariogenic properties of mutans streptococci *in vivo*.

The results of this study demonstrate that the level of mutans streptococci was significantly lower in the mouthrinse group than in the no mouthrinse group. Odds = 0.64 by logistic multiple regression analysis shows that the possibility of an increase in the level of bacteria by enforcing fluoride mouthrinse is 0.64 times higher. That is, the odds of a child who had performed fluoride mouthrinse having a low level of mutans streptococci is 1.56 times as high as the odds for a child who had not performed fluoride mouthrinse. In this study, the relationship between fluoride mouthrinse and the salivary level of mutans streptococci or lactobacilli was evaluated under conditions in which other factors were eliminated by using the multivariate statistical analysis. Even if the groups with low bacteria count had a higher rate of sealant on permanent teeth (Table 2), a significant relationship to the salivary level of mutans streptococci or lactobacilli was not recognized by logistic multiple regression analysis.

Furthermore, we evaluated the mean number of permanent tooth surfaces sealed by high and low level of mutans streptococci or lactobacilli in mouthrinse and no mouthrinse groups (Table 4). There was no significant difference between high and low salivary levels of mutans streptococci or lactobacilli by each age in both the mouthrinse and no mouthrinse groups. The only significant difference was for salivary levels of lactobacilli of no mouthrinse group in 10-year-olds. From these results, it was conceivable that the relationship between the sum of permanent tooth surfaces sealed and the salivary level of mutans streptococci or lactobacilli may be weak. Concerning the microbial effect of pit and fissure sealant with a resin based material, some reports have shown that after sealant application there might not be any significant differences in the salivary levels of mutans streptococci compared to the control group[10,16] These findings naturally suggest that the fluoride mouthrinse may have caused a reduction in the level of mutans streptococci.

A high level of fluoride in plaque was reported in the early 1960s. The concentration of fluoride measured in plaque was markedly higher than the corresponding value in saliva. It is important to differentiate the level of fluoride, which may occur *in vivo* as a result of some preventive therapy. All bacteria might be subject to different inhibitory effects as the fluoride concentration increases.

In a previous study, it was shown that the level of fluoride at concentrations as high as 21 ppm does not produce any obvious effects on the composition of supragingival plaque.[17] Patients using 1% sodium fluoride gel did not demonstrate any change in the distribution of lactobacilli.[18] In the presence of 115 ppm in plaque, mutans streptococci was eliminated in 10 of 30 patients and lactobacilli remained in all subjects.[18] Enolase activity in strains of mutans streptococci was inhibited by 50% by fluoride concentrations ranging from 16 to 54 microM.[19] On the other hand, in an *in vitro* study a mixed culture with 19 ppm fluoride did not decrease the level of lactobacilli.[6] Lactobacilli might have a good resistance to fluoride.

Based on these findings, it may be reasonable to conclude that the long-term use of fluoride mouthrinse, using 500 ppm or 2000 ppm sodium fluoride solution, restricted the level of mutans streptococci in the present study.

The salivary levels of mutans streptococci and lactobacilli were estimated with a screening method which might be a rapid and easy way to perform an on-site culturing method which is well tolerated by children.[15,20,21] Significant correlations to the conventional agar-plate method for determining the level of salivary mutans streptococci or lactobacilli have been found.[20]

In any event, we should note that the mean DMFS in the no-mouthrinse group was about 10 times as high as that in the mouthrinse group. Concerning the effectiveness of fluoride mouthrinse, some reports have shown that if children participated in fluoride mouthrinsing from 5-6 years old, the caries prevention rate was 55-80%.[4,22] The actions of fluoride in preventing caries might be multiple: enhanced remineralization, antimicrobial effect and modification of the physicochemical properties of teeth, etc. In this study, children who had a score of 3 for mutans streptococci had a higher tendency in mean DMFS than children who had a score of 0, but no significant differences were found. Moreover, there were significant, but slight differences in the distribution of mutans streptococci between the mouthrinse and no mouthrinse groups. These findings confirm that the antibacterial action of fluoride mouthrinse does not have a great effect on caries initiation as compared with the effects of other factors such as enhanced remineralization.[23]

Conclusion

In conclusion, therefore, the data presented here suggest that long term use of fluoride mouthrinse might have an effect in reducing the salivary level of mutans streptococci.

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