Mutans streptococci, lactobacilli in saliva and acidity from organismsin dental plaque: changes after restorative treatment

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The effect of restorative dental treatment was evaluated on mutans streptococci and lactobacilli in saliva, as well as acid production by plaque bacteria. We used semiquantitative culture kits at time points up to 6 months following treatment. Changes in eating habits and oral hygiene during the study period were ruled out using a questionnaire. Mutans streptococci, lactobacilli, and acidity all had decreased significantly at 1 week after treatment. Acidity often was the first variable to return to pretreatment levels, while abundant at 6 than 3 months. Lactobacilli showed the most durable response to treatment. Restorative treatment achieves import temporary decreases in caries-associated bacteria, especially lactobacilli, without influence from potentially relevant behavioral changes. In addition, follow up examinations at relatively long intervals at least 3 month would appear most effective for dental health management.

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INTRODUCTION

Until recently in Japan, the goals of primary and secondary prevention of oral diseases had followed the three phases and five stages of Leavell and Clark.[1] While these goals have been replaced by one of promoting ideal oral health, this optimal goal is not attainable in many districts where incidence of caries remains high and no improvement in addressing the problem of untreated caries has occurred. As a means of initiating oral health improvement in these districts, motivation for caries prevention and changes in dental health behavior, can be promoted during treatment. However, this educational role of treatment for prevention is generally overlooked.

Restorative treatment corresponds to secondary prevention, while prosthetic treatment corresponds to tertiary prevention among the three phases of prevention outlined by Leavell and Clark.[1] In this scheme, treatment is firmly positioned in the context of prevention.[1]

We interpret prevention broadly to include promoting behavioral changes that favor better oral health in a manner appropriate to the specific district, the individual, and the phase of prevention.Morinushi *et al*.[2] reported that intensive dental treatment in a preschool population in a district without dentist produced to not only an increase in the proportion of treated teeth, but also to a decrease in caries incidence. This program was initiated because the district had shown essentially no primary preventive effect over 18 years from long-term dental health instruction as generally performed in Japan, including a combination of oral examination, individual verbal instruction, and fluoridation.[2] These authors attributed the improvements with intensive treatment to changes in public attitudes concerning dental health, but changes in each individual were not sufficiently explained. Restorative treatment could improve oral health by a variety of mechanisms including changes in caries-related bacteria. Although there were several reports which restorative treatment have considered decrease in caries-related bacteria such as *Streptococcus mutans* and *Lactobacillus,* no report sufficiently considered a possible contribution from improved eating and tooth brushing habits.[3-6] To elucidate the preventive contribution of restorative treatment, we did a survey of acid production by bacteria in dental plaque, and colony counts of mutans streptococci and lactobacilli in saliva before treatment and up to 6 months after-ward, giving attention to eating and toothbrushing habits.

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Table 1. Subjects

	18 subjects initially included		Participation until 6 months after treatment		13 subjects for questionnaires	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Number	8.0	10.0	5.0	7.0	5.0	8.0
Mean age at first examination	4.5	4.5	4.8	4.7	4.4	4.5

MATERIALS, METHODS AND SUBJECTS

Subjects and Materials

The Institutional Review Committee of Kagoshima University Dental School approved all protocols involving human subjects, and the study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1983. Before participation subjects and their parents gave informed consent. The subjects were 18 pediatric outpatients aged 3 to 7 year (mean, 4.7 year) at our facility at Dental Hospital of Kagoshima University. All subjects had a decayed-missing-filled (dmf) index exceeding 40 with at least two active cavities (Table 1). The mean dmf-tooth number per person (the mean dmf-T) in the subjects was 54.7 ± 13.4, and the mean Caries Severity Index (CSI) (Figure 1) was 35.4 ± 12.34. Of the 18 subjects initially included, 12 continued participate until 6 months after treatment. Follow-up examinations are described below see Methods. The mean dmf-T in the 12 subjects was 57.57 ± 14.54, while the mean CSI was 35.84 ± 13.50.

A dentist examined caries using compressed air and number 23 explorers under artificial light. Additionally, bite-wing radiographs were obtained in all subjects. A pediatric dentist performed conventional restorative treatment of all caries detected by clinical and radiographic examinations. Treatment used mainly composite resin fillings. Vital pulpotomy and cementation of steel crowns were performed when necessary. The total number of treated teeth was 123, while the number of treated teeth per child was 10.3 ± 2.7. Of the l27 teeth in 12 subjects, 4 were extracted, and 30 had pulp treatment. Only fillings were used in the remaining 89. No occurrence of new or secondary caries was observed in any subjects until 6 months after initial treatment.

Methods

A CARIOSTAT kit (caries activity test, or CAT; Sankin, Tokyo) was used for evaluating the acid-producing ability of bacteria in dental plaque.[7-10] The buccal aspects of the maxillary teeth were wiped with a cotton swab provided, which was incubated in culture fluid for 24 h. At that time acidity was evaluated according to changes in the color of the culture fluid.[7-10]

Dentocult-SM strip mutans (DSM; Orion Diagnostica) and Dentocult-LB (DLB; Orion Diagnostica) kits were used to perform colony counts of mutans streptococci and lactobacilli respectively.[11-13] The test was performed according to a simplified method described by Morinushi *et al.*[14] Secretion of saliva was gently stimulated saliva without chewing paraffin according to a conventional method. Specimens were collected at four time points: before treatment, after treatment and at 3 and 6 months after treatment. For DSM, the collecting strips provided were rotated approximately 10 times on the dorsum of the tongue to moisten them sufficiently with saliva: the strips then were immersed in the culture fluid provided incubation at 37˚C for 48 h. The number of colonies on the strips was evaluated semi-quantitatively as representing one of four stages according to the kit protocol of the manufacturer. For DLB, the necessary amount of saliva was collected from the lingual side of the mandible at the most posterior molar, using a syringe. The saliva was inoculated on both sides of agar slides for incubation at 37˚C for 4 d. Development of colonies on the slides was rated according to five stages (absence of colonies being classified as 0) according to the manufacturer's protocol.

For 13 subjects, questionnaires regarding dental health behavior and past and present eating habits were collected before treatment and again at the 3-month follow-up examination. Changes in answers were evaluated. Although fluoridation was performed after treatment and at the time of specimen collection at follow-up examinations, instructions concerning toothbrushing and eating habits were not given, in order to minimize the influence of changes in dental health behavior on the results.

Analysis

Statistical evaluation of differences in scores stages between time points was performed using the non-parametric Wilcoxon signed-ranks test.

RESULTS

CAT results and treatment

The change of CAT value in each subject is seen in Figure 2. CAT values for acidity were decreased from

M-CSI = total score x 100 / (2 x total number of erupted teeth)

Scoring: healthy = 0; C0 = 0.5; Clor C2 = 1; C3 or C4 = 2, Secondary caries = 1.5; treated tooth = 1

*Severity of caries is evaluated by cavitation level.
C0: no cavity with demineralization in enamel,
Cl: cavity within enamel,
C2: cavity reaching to dentin,
C3; cavity reaching to pulp,
C4: toothcrown destroyed by caries.

Figure 1. M-CSI as defined by Shimono et al.[7]

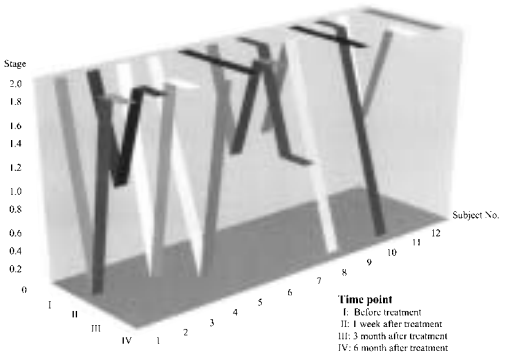

Figure 2. Change of CAT stage at four time points

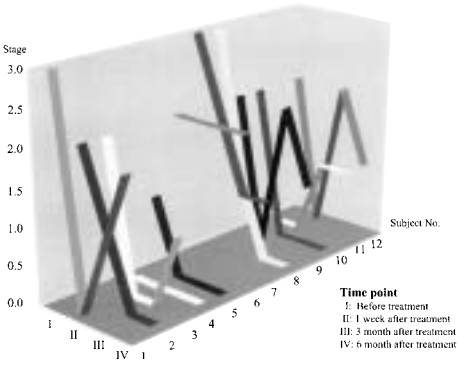

Figure 3. Changes ofDSM stage at four time points.

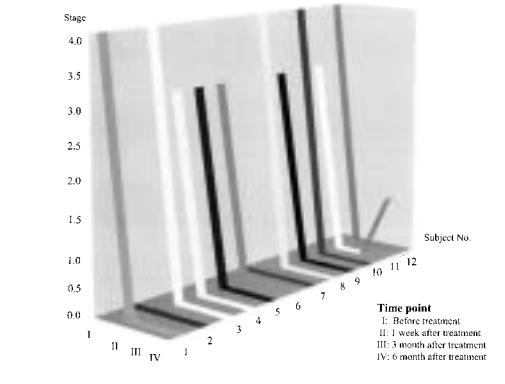

Figure 4. Changes ofDLB stage at four time points.

baseline at 1 wk after treatment in 6 subjects (50.0%). No change was observed in 5 subjects (41.7%)(2-2 in all), and deterioration increased acidity was observed in 1 subject (8.3%). Between 1 wk after and 3 wk, acidity was increased in 5 subjects (41.7%) while no change was noted in 3 subjects (25.0%) and acidity was decreased in 4 subjects (33.3%). Between 3 and 6 months after treatment, increased acidity was observed in 1 subject (8.3%), no change was noted in 9 subjects (75.0%), and a decreased in acidity-defined stage from 1 to 0 was observed in 2 subjects (16.7%).

DSM results and treatment

The change of DSM stage is seen in each subject in Figure 3. Improvement indicated by decreases of more than two in DSM stages between baseline and 1 wk after treatment was observed in 6 subjects (50.0%). Improvement by one stage was observed in 3 subjects (25.0%). Four subjects (25.0%) (0-0; two, 2-2; one) showed no change. Between 1 after treatment and 3 months after treatment, deterioration increases in DSM stage was observed in four subjects (33.3%); no change was noted in five subjects (41.7%), and improvement was observed in three subjects (25.0%). Between 3 and 6 months after treatment, deterioration increases in DSM stage, was observed in 2 subjects (16.7%); no changes was noted in 8 subjects (66.7%), and improvement was observed in two subjects (16.7%).

DLB results and treatment

The change of DLB stage in each subject is seen in Figure 4. Improvement indicated by a decrease of more than two DLB stages between baseline and 1 wk after treatment was observed in ten subjects (83.3%). Two subjects (16.7%) (0-0 in all) showed no change

Between 1 after treatment and 3 months after treatment, no change was noted in all of subjects (41.7%). Between 3 and 6 months after treatment, deterioration increases in DLB stage was observed in only one subject (8.3%); no change was noted in the remaining eleven subjects (91.7%).

Statistical evaluation of caries-associated bacterial markers

Comparison of results for between time points showed no significant differences between 1 wk after and 3 or 6 months after treatment. However, a significant difference for each test was noted before treatment and 1 wk after treatment; $p<0.05$, $z=2.013$ for CAT; $p<0.002$, $z=-3.247$ for DSM; and p0.00l, $z=-3.342$ for DLB. Moreover, significant differences were observed before treatment and 3 months or 6 months after treatment for DSM ($p<0.05$, $z=-2.372$ and $p<0.05$, $z=-2.511$) and for DLB ($p<0.01$, $z=-2.879$ and $p<0.05$, $z=2.157$), but not for CAT.

Questionnaires

According to questionnaires, eight of the thirteen subjects (61.5%) used feeding bottles to drink commercially available juices and six subjects (46.2%) used these bottles to consume commercially available sports drinks prior to the age of 18 months. Intake of sweet beverages with feeding bottles before sleeping was noted in six subjects (46.2%). Parents assisted on toothbrushing prior to the age of 3 years for 12 subjects (92.3%). Between 1 wk after and 3 months after treatment, no changes occurred in the presence of food intake before sleeping, and no change occurred concerning parental assistance with toothbrushing for any subjects. No change occurred in the other three items (frequency of meals, regularity of mealtimes, and frequency of between-meal snacks) for 92.3% of subjects.

DISCUSSION

By evaluating treatment-associated changes in indices of abundance of caries-related bacteria while ruling out changes in relevant eating and tooth-brushing habits, we demonstrated a preventive effect of treatment against occurrence of caries and showed a relationship between caries and two bacteria. We also established an appropriate interval for follow-up examinations by determining the relapse period indicated by changes in amounts caries-related bacteria.

In our comparisons of CAT, DSM, and DLB between before treatment and 1 wk after completion of treatment, 50.0% of subjects showed decreases in CAT, indicating decreased ability of bacteria 1 specimen collected from supra-gingival dental plaque to produce acid. In 75.0% of subjects, DSM showed decreases in the number of colonies grown from a sample of saliva from the dorsum of the tongue. In DLB, 83.3% of subjects showed decreases in the number of colonies grown from a sample of gently simulated saliva. Decreases in numbers of colonies by at least two stages were observed in 50.0% of subjects for DSM and in 83.3% subjects for DLB, showing that treatment decreased lactobacilli more than mutans streptococci Differences in results before and 1 wk after treatment were statistically significant for all three caries activity markers, especially DLB ($p<0.005$).

On the other hard, changes in acid production bacteria in dental plaque (CAT) with treatment were smaller than decreases in mutans streptococci and lactobacilli in salivary specimens.

Keene *et al*.[3] reported that fewer *S. mutans* organisms were detected on tooth surfaces without caries than on surfaces with caries, and that significant decreases in detection rate on tooth surfaces with caries resulted from treatment. Wright *et al*.[4] reported that although both *S. mutans* and *Lactobacillus* decreased with treatment, these bacteria regained the previous levels after 151 days in 50% of subjects. These authors also reported a close relationship between the two bacteria and caries, with a decreased risk of developing caries following treatment, at least temporarily.[4] Petti *et al*.[5] compared detection of *S. mutans* in saliva as well as colony counts among between three groups: group 1 had sound teeth, while complete treatment of decayed teeth had been carried out in group 2 but group 3 had untreated decayed teeth.[5] Group 2 showed significantly lower values than group 3; no differences were observed between groups 1 and 2. These authors also suggested that the risk of developing caries was decreased by treatment via decreases in *S. mutans*.[5] Our present study, it suggested that additionally acid production ability in dental plaque (CAT) decreased after treatment, lactobacilli, which was highly associated with acid production and caries cavity, showed the greatest decrease with treatment.

In contrast, Gregory *et al*.[6] reported that the number of *S. mutans* colonies in 12 subjects decreased only insignificantly following treatment according to categorized mean values. Notably, however, 50% of these subjects showed decreases in the number of colonies, while 33.3% of subjects showed no change, and 16% showed increases. Thus, the number of subjects, in whom *S. mutans* was decreased by treatment, was relatively large. Furthermore, these evaluations were expressed categorically, but the parametric Student / test was used for statistical evaluation. Therefore, the results of Gregory *et al*.[6] do not appear to differ from other reported findings.

Concerning the time course of changes after treatment, Wright *et al*.[4] reported that both *S. mutans* and *Lactobacillus* recovered their previous levels after 151 days in 50% of subjects. However, no data concerning possible charges in eating habits or dental health matters were considered in their report. Frequency of between-meal snacks, intake of sweet beverages, intake of sucrose, and frequency of meals have been reported to correlate positively with detection and amounts of *S. mutans* and *Lactobacillus*.[15-18] We confirmed that no changes in eating habits or dental health behavior had occurred, at least at 3 months after treatment. We therefore consider our results to be highly reliable.

In our study, CAT, DSM, and DLB all showed significantly lower values at 1 wk after treatment than

before treatment. However, CAT showed no significant differences between pretreatment findings and those at or 6 months. CAT showed a tendency to relapse in 75% of subjects at 3 months after treatment. Although DSM showed significantly lower values both 3 and 6 months after treatment in comparison with pretreatment findings, relapse was observed 3 months after treatment in 25.0% of subjects. In contrast with CAT and DSM, DLB showed significantly lower values at both 3 and 6 months after treatment than before treatment, while no subject showed a relapse at 3 months after treatment. These results suggested that *Lactobacillus*, which is closely related to caries activity, was greatly influenced by treatment (Table 2). A decrease of *Lactobacillus* related to treatment has been observed consistently during a period with no occurrence of new or secondary caries.[19] In contrast, influences of treatment on other acidogenic bacteria present in dental plaque that are clinically related to caries incidence were small and relatively transient. Mutans streptococci (DSM) showed intermediate results in comparison with CAT and DLB. A tendency to relapse was noted at 3 months after treatment, while also was suggested by Wright *et al*.[4]

Table 2. Maintenance of improvement at 3 and 6 months after treatment.

Test	After 3 months	After 6 months
CAT	3 (25.0%)	3 (25.0%)
DSM	9 (75.0%)	10 (83.3%)
DLB	12 (100.0%)	12 (100.0%)

We made this table by participation (twelve subjects) until 6 months after treatment. We defined change from stage 0 to 0 as maintenance of improvement.

M-CSI = total score x 100 / (2 x total number of erupted teeth)
Scoring: healthy; C0=0.5; Clor C2-1; C3or C4=2,
Secondary caries =1.5; treated tooth-1

*Seventy of caries is evaluated by cavitation level.
C0: no cavity with demineralization in enamel,
CI: cavity within enamel,
C2: cavity reaching to dentin,
C3; cavity reaching to pulp,
C4: toothcrown destroyed by caries.

For several reasons, caries markers were evaluated in our study, as opposed to methods used in other reports. First, these tests can be performed at chairside, so practitioners can directly apply the results. Second, conditions of specimen collection had relatively little influence on results. The stages evaluated were broad categories, enhancing reliability. Furthermore, although detection rates by DSM and DLB were lower than for the conventional saliva collection method including paraffin chewing, the accuracy of evaluation became higher, and differences in the results became more durable, since saliva specimens were less likely to contain dental plaque from tooth surfaces.

CONCLUSION

These results suggested that susceptibility to caries were temporarily lowered by restorative treatment as shown by decreases in caries-related bacteria. Follow up examinations at relatively long intervals at least 3 month would appear most effective for dental health management.

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