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Effect of sodium hypochlorite solution on the subgingival microflora of juvenile periodontitis lesions

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Abstract

The effect of gingival curettage facilitated by sodium hypochlorite solution on the subgingival microflora of 21 juvenile periodontitis lesions was evaluated. Dark-field analysis and anaerobic culturing of juvenile periodontitis lesions were made immediately prior to treatment with gingival curettage facilitated by sodium hypochlorite solution— immediately after therapy, 30 days post-therapy, and 90 days post-therapy. Results indicate that the therapy is capable of significantly altering the quantity and quality of subgingival organisms found in the juvenile periodontitis lesion and maintaining this alteration over a 30- to 90-day period.

Localized, advanced periodontal destruction in the molar and incisor regions of the adolescent dentition has been known by many different names: diffuse atrophy, periodontosis, juvenile periodontitis, and precocious periodontitis. Because of the different names and diagnostic criteria, there has been much confusion regarding this condition. In the recent literature, two clinical terms have been predominated: periodontosis and juvenile periodontitis. Juvenile periodontitis may be defined as a severe, inflammatory disease of the periodontium characterized by a rapid, localized loss of alveolar bone around the secondary incisors and first molars in young individuals.[1]

In recent investigations, a unique microbial flora has been shown to be associated with juvenile periodontitis. In 1974, Sumney and coworkers[2] demonstrated little plaque accumulation on the root surfaces affected by juvenile periodontitis as well as a prominence of gram-negative bacteria. In 1975 a group of investigators[3,4] described patterns of gram-negative rod colonization in juvenile periodontitis lesions and characterized a fusiform bacteria. In 1976 Listgarten[5] studied the microflora associated with periodontal health and diseases in man. He reported that patients with juvenile periodontitis lacked visible surface deposits.

Newman and coworkers[6] categorized an increase in gram-negative rods in the flora of juvenile periodontitis. Newman and Socransky[7] later reported that bacterial samples obtained from juvenile periodontitis lesions contained increased portions of gram-negative anaerobic rods and a significant number of *Capnocytophaga* species. Slots[8] also found that the microflora of juvenile periodontitis was dominated by gram-negative anaerobic rods. In 1979 three separate studies found *Actinobacillus actinomycetemcomitans* in high proportions in juvenile periodontitis pockets.[9-11] Recent investigations have reported invasion of bacteria into the connective tissue adjacent to deep periodontal pockets.[12,13] One report has demonstrated a similar tissue invasion associated with a juvenile periodontitis lesion.[14]

A few studies have evaluated the effect of nonsurgical periodontal therapy on the bacterial flora of juvenile periodontitis lesions. Genco and coworkers[15,16] concluded that scaling and root planing alone often were not capable of reducing the numbers of pathogenic organisms in juvenile periodontitis cases. In a recent study, Slots and coworkers[17] demonstrated that scaling and root planing (averaging six hours of debridement) were incapable of eliminating *Capnocytophaga* and *A. actinomycetemcomitans* from juvenile periodontitis lesions.

Gingival curettage facilitated by sodium hypochlorite solution has been investigated.[18-20] The early objective of using sodium hypochlorite solution to aid in curettage was to enhance removal of inflamed soft tissue.[18] The solution has been shown to be bacteriocidal and has no detrimental effects on soft tissue healing.[19-20]

Since isolated lesions characteristic of juvenile periodontitis are populated by a unique flora and traditional nonsurgical periodontal therapy has been shown to be ineffective in altering this flora, this study was designed to investigate the antimicrobial effects of sodium hypochlorite solution on the subgingival microflora associated with juvenile periodontitis.

Methods and Materials

Patients selected for this protocol met these criteria:

1. Less than 18 years of age
2. Clinical and radiographic evidence of juvenile periodontitis (localized 6 mm or greater probing depth and bone loss around first molars and/or incisors)
3. No periodontal therapy, including prophylaxis or plaque control instruction, within the past six months
4. No evidence of a systemic disturbance as diabetes, hematologic problems, or connective tissue disturbances which might influence the course of the juvenile periodontitis or possible therapeutic approaches
5. No antibiotic therapy within the previous six months.

Eleven patients originally met the criteria for patient selection, eight of whom were from one family. (As the investigation progressed, one patient dropped out.) The patients ranged in age from 7 to 17 years. The onset of juvenile periodontitis usually is associated with puberty, but siblings aged 7 and 10 from the family of eight exhibited both clinical and radiographic evidence of juvenile periodontitis, and were included in this study.

One to three molars and/or incisors exhibiting clinical and radiographic evidence of the disease were identified as experimental teeth in each patient. A total of 22 experimental sites were identified. One control tooth exhibiting no clinical evidence of periodontal disease was chosen in each patient. The control region served to evaluate the effect of sodium hypochlorite solution on the bacteria associated with a shallow sulcus. Comparison of post-treatment samples between the experimental and control regions would parallel each other if there were an antibacterial effect of the solution.

Initial bacterial samples were obtained from the gingival crevice using an instrument modified from reports by Newman and Socransky.[7] A syringe was modified so oxygen-free argon gas could be passed through an attached 20-gauge cannula, 4 mm long (Figure 1). The plunger region was modified to permit passage of the barbed broach soldered to a long stainless steel wire. The barbed broach was wound with sterile cotton fibers. In use, the tip of the cannula, capped with tinfoil to prevent contamination of supragingival plaque, was inserted into the pocket. The broach then was passed down the cannula, dislodging the foil cap. The broach then scraped the bottom of the pocket and adjacent tooth surfaces, the fibers trapping loose organisms. The barbed broach was withdrawn into the cannula and the entire apparatus was removed. The tinfoil cap later was removed from the gingival crevice using a scaler. Once the sample was obtained, the broach was introduced into a prereduced anaerobically sterilized medium containing tryptic soy broth and thioglycolate maintained in an argon environment. (The addition of thioglycolate allowed the broth to function as a transport media.)

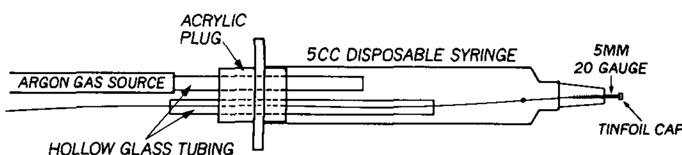

Figure 1. Diagrammatic representation of microbial sampling apparatus.

The sample was dispersed by placing a cavitron tip on the stainless steel wire approximately four centimeters above the broach and running the instrument at high power without water spray for 5 seconds. Five seconds of vibration allowed dispersion of the organisms from the broach without affecting viability of the sonic-sensitive spirochetes. While some organisms may have become entrapped within the cotton fibers and not totally dispersed by the vibration, the effect was uniform in all phases of the study.

The sample solutions were evaluated with dark-field microscopy utilizing 0.2 ml of the dispersed solution. The solution was placed on a precleaned glass slide, covered with a glass cover slip and immediately examined with a microscope equipped with a dark-field condenser, 95x dark-field objective, and a 10x eyepiece. Bacteria were identified and placed into four groups:

1. Spirochete — coiled-appearing
2. Fusiform — tapered ends with a ratio of 8:1; length to width
3. Rod — straight or curved
4. Coccus — length exceeding diameter by no more than 2:1
5. Other.

At least 100 bacteria were identified from 10 fields.

After the samples were dispersed, they were transferred into an anaerobic tent for plating. Plating was accomplished on tryptic soy agar plates made selective for gram-negative bacteria by the addition of vancomycin and menadione.[21] (Menadione was included to support the growth of bacteroides, common in advanced periodontal defects.) Two plates were prepared from each sample by placing and spreading two drops of the dispersed solution on the surface of the plate. The plates were grown in anaerobic jars in a 90% nitrogen and 10% carbon dioxide environment.

After three days of growth, the colony types were differentiated by color, texture, surface characteristics, etc. Each different colony type was streak-isolated on fresh plates and incubated anaerobically in the environment described previously. After pure isolates were obtained, portions were grown aerobically to determine which isolates were facultative. The isolates were gram-stained using normal techniques. The gram stains were evaluated

for determination of gram-negative or gram-positive characteristics, morphology, and size of the bacteria. The size of the bacteria was determined using a microscope with an ocular grid.

Following initial sampling, both experimental and control sites were subjected to sodium hypochlorite-gingival curettage according to the technique described by Kalkwarf and coworkers[19] with minor changes. Scaling and root planing were not completed in advance of treatment and only inadvertent scaling was done during treatment. Scaling and root planing were avoided so that alterations in flora could be attributed to the sodium hypochlorite-facilitated gingival curettage. Sodium hypochlorite solution (pH 13.95) was introduced to the depth of the pocket with a No. 2 medicament loop, and was allowed to remain undisturbed for 90 seconds. Application of several drops of 5% citric acid solution neutralized the region and controlled time of action. Removal of the soft tissue lining of the pocket then was performed with six strokes of a sharp periodontal curette. Bacterial samples were obtained immediately following gingival curettage in the manner previously described, and dark-field microscopy and plating techniques were repeated. No dressing was placed following curettage and patients were given no specific postoperative directions other than to return to their normal plaque control methods the following day. Patients were recalled at 30 and 90 days for bacterial sampling.

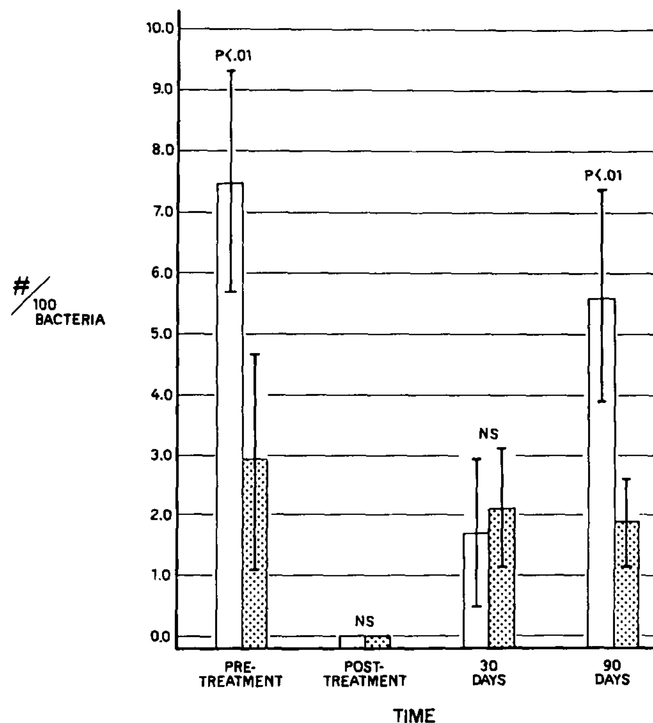

Figure 2. Mean proportions of spirochetes in dark-field evaluations; clear column-experimental sites; shaded column-control sites.

Results

Dark-Field Microscopy

Other than in the immediate post-treatment sample which exhibited no organisms, the proportions of cocci and rods remained stable in all samples. Proportions of spirochetes and fusiform bacteria found during the dark-field evaluation of the dispersed samples are shown in Figures 2 & 3. Figure 2 shows the mean percentage of spirochetes present at the sampling intervals while Figure 3 represents fusiform bacteria present in the samples. Table 1 shows the change in subgingival flora through the treatment and post-treatment observation period. Differences of post-treatment counts from pretreatment counts were analyzed by Student's t-distribution.

Dark-field evaluation shows that sodium hypochlorite is capable of eliminating all spirochetes and fusiform bacteria from the subgingival environment. Gradual rises in populations over the 90-day postoperative period results in spirochete levels below those exhibited prior to treatment and fusiform bacteria levels above those shown before therapy.

Plating Results

Morphologic identifications were made of gram-negative anaerobic bacteria present in the crevice areas about experimental and control teeth. Table 2 shows the percentage of teeth exhibiting specific gram-negative anaerobic bacteria in their crevice areas. Differences in individual anaerobic bacteria between experimental and control groups are evident at the various culturing intervals, but are not statistically significant due to the heterogeneity.

Discussion

Figure 2 shows that at 30 days there is a net decrease in the number of spirochetes compared to pretreatment levels. As the plaque begins to mature between 30 and 90 days, spirochete numbers increase.

Figures 2 & 3 are combinations of data acquired from the individual teeth evaluated. There is a possibility that

Table 1. Comparison of Post-treatment Dark-Field Bacterial Counts With Pretreatment Counts

	Experimental Teeth (21)	
	Spirochetes	*Fusiform Bacteria*
Pretreatment	7.5 ± 3.6	2.25 ± 1.71
Immediately post-treatment	0.0 ± 0.0 p<.01	0.0 ± 0.0 p<.01
30 days post-treatment	1.7 ± 2.3 p<.01	2.4 ± 1.9 p>.05 NS
90 days post-treatment	5.6 ± 3.6 p<.05	6.0 ± 2.8 p<.01
	Control Teeth (10)	
	Spirochetes	*Fusiform Bacteria*
Pretreatment	2.9 ± 3.6	1.2 ± 1.75
Immediately post-treatment	0.0 ± 0.0 p<.01	0.0 ± 0.0 p<.01
30 days post-treatment	2.1 ± 2.0 p>.05 NS	3.5 ± 1.9 p<.01
90 days post-treatment	1.9 ± 1.3 p>.05 NS	4.0 ± 2.1 p<.01

not all teeth sampled were active juvenile periodontitis lesions and, thus, a dilution of the true numbers of significant bacteria could be evident. The age group of the population and the clinical appearance of the lesions lead one to believe that the majority of lesions were active.

Scaling, root planing, and oral hygiene instruction were not given to patients since the purpose of this study was to determine the effects of sodium hypochlorite-citric acid solutions. As has been pointed out by Slots and coworkers,[17] scaling and root planing could be responsible for alterations in microflora. Because of this, some

Table 2. Percentage of Teeth Exhibiting Specific Gram-negative Anaerobic Bacteria

	Experimental (21 teeth)				Control (10 teeth)			
	Pre-	Post-	30	90	Pre-	Post-	30	90
1. Pleomorphic Rod 4.8 x 0.8 μ	71	19	62	76	70	30	60	80
2. Curved Rod 1.2 x 0.4 μ	19	5	10	10	40	0	30	0
3. Pleomorphic Rod 1.0 x 0.4 μ	10	0	0	5	10	0	20	20
4. Cocci 0.6 μ	43	10	33	24	50	20	20	20
5. Straight Rod 1.2 x 0.6 μ	10	0	14	14	0	0	0	0
6. Straight Rod 0.8 x 0.2 μ	62	5	19	10	0	0	20	0
7. Cocci 1.0 μ	43	14	10	0	20	10	0	0
8. Pleomorphic Rods 2.8 x 0.6 μ	43	5	24	14	0	0	20	10
9. Straights Rods pale staining 3.6 x 0.6 μ	38	14	19	38	30	0	20	70
10. Cocci 0.3 μ	19	19	14	33	0	0	0	0
11. Straight Rods 2.4 x 0.6 μ	0	0	0	38	30	10	20	20
12. Straight Rods 0.2 x 0.4 μ	0	0	0	0	10	0	10	0
13. Filamentous Rods 7.2 x 0.6 μ	24	0	0	14	0	0	0	40
14. Filamentous Rods 4.8 x 0.6 μ	14	0	0	19	0	0	0	0

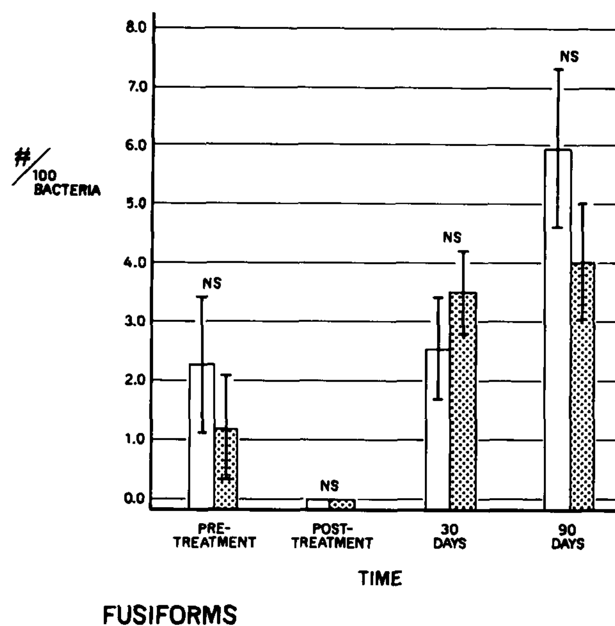

Figure 3. Mean proportion of fusiform bacteria in dark-field evaluations; clear column-experimental sites; shaded column-control sites.

observed effect may have been due to inadvertent scaling occurring during curettage. Since home care instruction was not given to the patients, there were varying amounts of supragingival plaque noted in both control and experiment sites at the 30-day and 90-day examinations. Participating in a study that sampled bacteria may have had a positive effect on oral hygiene habits through the course of the study — this could be particularly true in the family group that participated.

Culturing gram-negative anaerobes was done to evaluate a qualitative change in the bacterial plaque following treatment. Only gram-negative anaerobes were included because of increasing evidence linking this class of bacteria with the etiology of juvenile periodontitis. The results of the culturing procedure showed an alteration of several bacterial types 30 and 90 days following treatment and a difference between experimental pockets and control sulci.

Examining individual sites revealed some variations from the general trend of data. Immediately following treatment, it was possible to culture gram-negative anaerobes from a 12 mm pocket involving the facial furcation of a maxillary first molar. Because of the difficulty in treating such a region with sodium hypochlorite administered via a loop, new methods of delivery such as irrigating syringes should be evaluated.[22]

The results of the experiment have shown that there is a decrease in the number of gram-negative anaerobic bacteria immediately following gingival curettage facilitated by sodium hypochlorite solution. This effect may be explained due to two possible mechanisms of action: (1) bacteria may be destroyed by direct bacteriocidal action of sodium hypochlorite solution, and (2) me-

chanical entrapment of bacteria may occur in the soft tissue which is denatured following placement of sodium hypochlorite solution. The bacteria then could be removed during curettement of this mass. Bacteria that have invaded the soft tissue also would be removed in this process.

From the results of the dark-field microscopy, there appears to be an increase in the number of fusiform bacteria present. Since there is a qualitative difference between a juvenile periodontitis plaque and an adult periodontitis plaque, this increase in Fusobacteria may indicate that the plaque was changed from a juvenile periodontitis plaque to an adult periodontitis plaque.[6]

Conclusions

Results of the experiment led to the following conclusions.

1. Gingival curettage faciliated by sodium hypochlorite solution alters the microbial flora of the juvenile periodontitis pocket.
2. A qualitative alteration of the plaque occurs following treatment, resulting in a net decrease in the number of spirochetes present.
3. Bacterial plaque is altered from 30 to 90 days following gingival curettage facilitated by sodium hypochlorite.

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