Using an Antibiotic Rinse System

An Adjunct in the Treatment of Periodontal Disease

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ABSTRACT
Research has now firmly established that dental plaque should be thought of as a biofilm and that periodontitis is a biofilm associated disease. The loss of a healthy balance in the microflora of subgingival tissues appears an important determination in the development of periodontitis. The structure and behavior of biofilm is what makes treatment of these infections more difficult. It has been found that the minimal inhibitory concentration dose for antibiotics and antimicrobials for bacteria in biofilm may be 20 to 200 times higher than required for free floating or plankton bacteria. Successful management of periodontal disease requires knowledge of oral bacteria and their behavior within a biofilm. Combining full scaling and root planing with an antibiotic can result in considerable improvement consisting of an increase in normal or shallow periodontal pockets and a decrease in the number of deep periodontal pockets. Fourteen patients, eight men and six women, with refractory periodontitis were selected randomly from a periodontal practice. Microbiology samples were taken from the throat, tongue and all four quadrants. After sampling was completed, each patient had the teeth cleaned, then was given a preparation of metronidazole, nystatin and water and told to rinse three times a day for 30 seconds. They were to continue this routine for two weeks and then return to the office for a follow-up appointment. All the tests were repeated at the end of two weeks and the results compared to baseline measurements. Only 10 patients completed all the periodontal measurements and nine patients have a complete pre and post treatment microbiology report. The results provided strong evidence that an antibiotic mouth rinse containing metronidazole and nystatin is effective in the treatment of periodontitis. Gingival tissues were healthier, with fewer bleeding points and a decrease in periodontal pocket depth. This system fits within the guidelines of the American Academy of Periodontology. The results indicate that a rinse system provides a beneficial adjunct in the treatment of periodontal disease.

Traditionally, periodontal diseases have been defined as plaque-induced and divided into two general categories; gingivitis, the inflammation of gingival tissues without attachment loss and periodontitis, an inflammatory response along with the pathological loss of collagen fibres from cementum and the junctional epithelium.[1] Recurrent, chronic or refractory periodontitis refers to progression of disease and loss of attachment despite clinical treatment.[2] This progression is often related to aggressive or persistent subgingival pathogens and possibly to impaired host resistance.[3]

Research has now firmly established that dental plaque should be thought of as a biofilm and that periodontitis should be considered a biofilm associated disease. The structure and behavior of biofilms is what makes treatment of these infections more difficult.[4] As the tooth surface becomes colonized, a sticky extracellular substance consisting of polysaccharides, proteins, lipids, nucleic acids and other polymers is secreted to help these bacteria adhere to the surface, as well as to each other. It has been found that the minimal inhibitory concentration dose for antibiotics and antimicrobials for bacteria in

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biofilms may be 20 to 200 times higher than required for free floating or plankton bacteria. This may be due to the difficulty in penetrating the sticky extracellular matrix. There is also a possibility that some of the bacteria found within the biofilm are less susceptible to antibiotics[4] and to antimicrobials.

In many cases, gingivitis and periodontitis can be controlled with good oral hygiene and regular professional cleaning. Scaling, polishing, and curettage in a series of three to four visits are used to manage periodontal disease. For those patients that respond to debridement with clinical improvement, there is a reduction in the levels of bacteria but if the biofilm is not disrupted frequently, these bacteria return to the predebridement levels.[5] Some organisms such as Porphyromonas gingivalis may persist because the depth of the pocket does not allow thorough debridement.[5] Surgery may be necessary to allow access for deep cleaning of the root surface, removal of diseased tissue, and repositioning and shaping of the bones, gum, and tissues supporting the teeth. Surgical procedures vary depending on the individual diagnosis and needs of the patient.

Successful management of periodontal disease requires knowledge of oral bacteria and their behavior within a biofilm. The loss of a healthy balance in the microflora of subgingival tissues appears an important determination in the development of periodontitis.[6] Several complexes have been associated with periodontal disease. Prevotella intermedia, Fusobacterium nucleatum and other bacteria found in the orange complex are associated with periodontal disease.[4] As the disease progresses, the periodontal pathogens of the red complex appear — Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola.[4] The parasite Entamoeba gingivalis is commonly found in patients with periodontal disease and is often seen in a background of marked inflammation with abundant neutrophils.[7] This parasite seems to be associated mainly with diseased gingival pocket sites.[8]

An important feature of oral bacteria is the ability to interact by co-aggregation, a recognition that occurs between genetically distinct bacteria types and includes physical contact, metabolic and genetic material exchange and signal communication.[9] As genetic exchange continues, the biofilm becomes less synergistic and more antagonistic with the most harmful and aggressive pathogens safe within the matrix. This change in the microflora of subgingival tissues is important in the development of periodontitis and can aid in future treatment decisions. Oral microbiology assessment can serve as a rapid diagnostic tool for detecting early stage periodontitis, as well as an aid in future treatment decisions.[6]

Oral microbiology assessment can serve as a rapid diagnostic tool for detecting early stage periodontitis

The American Academy of Periodontology Parameter on 'Refractory' Periodontitis outlines the following steps for diagnosis and treatment; "Once the diagnosis of refractory periodontitis has been made, the following steps may be taken:

1. Collection of subgingival microbial samples from selected sites for analyses, possibly including antibiotic-sensitivity testing.
2. Selection and administration of an appropriate antibiotic regimen.
3. In conjunction with the administration of an antimicrobial regimen, conventional periodontal therapies may be used.
4. Reevaluation with microbiological testing as indicated.[2]

The American Academy of Periodontology recommends re-evaluation with microbiological testing at one to three months after antimicrobial therapy. The rationale is that it may be desirable to verify the elimination or marked suppression of the putative pathogens and to screen for possible superinfecting organisms including Gram-negative enteric rods, psuedomonads and yeasts. Microbiological analysis is most effective when performed in sites that have not had any instrumentation. It usually takes four to eight weeks for pathogens to repopulate to pretreatment levels.[2] Microbial diagnosis should be considered in early-onset periodontitis or in subjects who respond poorly to conventional therapy. Microbial tests should then be applied to monitor the efficacy of mechanical treatment, as well as antimicrobial chemotherapy and to determine the end-point of active treatment.[10]

Table 1: Comparison of bleeding points

	PRE TREATMENT		POST TREATMENT	
	Bleeding Points		Bleeding Points	
Patient	Maxillary	Mandibular	Maxillary	Mandibular
RC	53	53	32	34
JS	34	40	34	34
KW	21	29	11	9
GF	32	51	14	14
SY	55	50	25	19
PD	34	16	18	12
EP	21	16	6	7
KG	42	54	14	32
MT	23	32	12	11
SH	13	17	9	13
	328	**358**	**175**	**185**
Difference	**153**	**173**		
% change	**46.64%**	**48.32%**		

Comparison of bleeding points at baseline and after two weeks of rinsing with an antibiotic solution.

The choice of treatment may be with an antibiotic such as metronidazole in conjunction with scaling and root planing and may result in statistically significant improvement in clinical attachment levels.[3] Metronidazole may be an effective choice as it shows activity against anaerobic cocci, gram-negative bacilli and gram-positive bacilli and its effectiveness has been demonstrated in severe adult and refractory periodontitis.[11]

The right choice of antimicrobial therapy is important and should be based on the pathogens present and linked with periodontal destruction.[12] Combining full scaling and root planing with an antibiotic can result in considerable improvement consisting of an increase in normal or shallow periodontal pockets and a decrease in the number of deep periodontal pockets.[13] Antibiotic therapy can reinforce mechanical treatment and support host defenses in overcoming periodontal infections by killing subgingival pathogens that remain after periodontal instrumentation.[4] Those pathogens that escape mechanical debridement because of their ability to invade periodontal tissues are more accessible with antibiotic therapy[3] that is applied locally or administered systemically.[4]

The objective of this research project was to determine if an antibiotic rinse preparation of metronidazole and nystatin would have an effect on the periodontal disease status of chronic (refractory) periodontitis patients. The second objective was to determine if the antibiotic rinse would decrease the pathogens present in the oral biofilm.

Method

Fourteen patients, eight men and six women, with refractory periodontitis were selected randomly from a periodontal practice. These

patients had been surgically treated and were now in the recare phase of treatment but continued to exhibit bleeding on probing and pocket depth > 4 mm. Each patient was examined for tissue changes, bleeding on probing and pocket depth using the six point evaluation (distal, centre, mesial) on both facial and lingual surfaces to establish a baseline. In addition, microbiology samples were taken from the throat, tongue and all four quadrants. Samples from the throat were taken using a flat mirror and placing the sample on a glass slide. The quadrants were sampled using a Soft-Pick for each sample and placing the tip deep into the pocket. The slides were sent to a microbiology lab for Gram-staining. The reports included both Gram-positive and Gram-negative bacteria, spirochetes, vibrio, yeast and the number of polymorphonuclear leukocytes and recorded as white blood cells (WBC).

Separate slides were taken of the four quadrants and were stained with Geimsa staining and examined for amoeboid-like structures.

After sampling was completed, each patient had the teeth cleaned. These patients were then given a preparation of metronidazole, nystatin and water and were told to rinse three times a day for 30 seconds. The patients were to swish vigorously for 20 seconds and gargle for 10 seconds then to floss immediately after rinsing at least once a day. They were to continue this routine for two weeks and then return to the office for a follow-up appointment. At this appointment, all the tests were repeated, including the microbiology samples, and the results compared to baseline measurements. Only 10 patients completed all the periodontal measurements and nine patients have a complete pre and post treatment microbiology report. Seven of the 14 patients showed amoeboid-like structures in the pretreatment analysis, but only three of these completed the full study.

Table 2: Decrease in Bleeding Points

DECREASE IN BLEEDING POINTS	MAXILLARY ARCH	MANDIBULAR ARCH
RC	39.62%	35.84%
JS	0.00%	17.64%
KW	46.61%	68.96%
GF	56.25%	72.54%
SY	54.54%	62.00%
PD	47.05%	25.00%
EP	71.42%	56.25%
KG	66.66%	40.47%
MT	47.82%	65.62%
SH	30.76%	25.52%

Percent change in bleeding points for each patient.

Table 3: Paired T-Test Analysis

	DEGREES OF FREEDOM	T =	P-VALUE
Maxillary BOP	df = 8	-5.050	P < 0.001
Mandibular BOP	df= 8	-4.794	P < 0.001
4 mm pockets	df = 8	-3.681	P < 0.01
5 mm pockets	df = 7	-3.100	P < 0.05
6 mm pockets	df = 6	-3.194	P < 0.05
7 mm pockets	df =1	-2	not significant
8 mm pockets	df = 3	-2.210	P <0.10

Paired Samples T-Test analysis in SPSS (Laerd – online calculator).
BOP=bleeding on probing.

Results

The comparison of total bleeding points at pre treatment and post treatment for maxil-

Table 4: Change in Number of Pockets Pre and Post Treatment

	POCKETS — PRE-TREATMENT					POCKETS — POST-TREATMENT				
	8mm	7mm	6mm	5mm	4mm	8mm	7mm	6mm	5mm	4mm
RC	1	0	8	18	28	1	0	0	7	15
JS	0	1	3	5	13	0	1	0	3	5
KW	0	1	3	9	14	0	1	0	1	8
GF	6	0	7	3	13	5	1	2	5	14
SY	0	0	0	0	3	0	0	0	0	0
PD	1	0	1	7	16	0	0	0	3	4
EP	0	0	1	11	12	0	0	0	0	8
KG	0	1	1	4	11	0	0	1	3	8
MT	0	0	3	4	6	0	0	2	3	2
SH	0	0	0	2	3	0	0	0	0	3
Total	**8**	**3**	**27**	**63**	**119**	**6**	**3**	**5**	**25**	**67**
Difference	**2**	**0**	**24**	**38**	**52**					
	25.00%	**0%**	**81.48%**	**60.31%**	**43.69%**					

Comparison of periodontal pocket depth at baseline and after two weeks of antibiotic rinsing.

lary and mandibular bleeding points showed a change of 46.64% on the maxillary arch and 48.32% on the mandibular arch (Table 1). Every participant had a positive change but the changes varied with each individual (Table 2). A paired T-Test was performed to ascertain whether the antibiotic rinsing was effective (Table 3). There is evidence that there was a substantial decrease in bleeding on probing and that it was statistically significant at a $p < 0.001$.

When the periodontal pocket measurements were compared, there were overall changes in the number of pockets for each category of measurements except for 7 mm pockets. These changes were as follows; 8 mm pockets had a 25% change; 81.48% change for 6mm pockets; 60.41 % change for 5 mm pockets and a 43.69% change for 4mm pockets (Table 4). Post treatment examination showed that patient GF, with six 8 mm pockets, now had five 8mm pockets and one 7 mm pocket. Patient PD had one 8 mm pocket pretreatment and the pocket measured 5 mm post treatment. Two patients (JS & KW) had no change in their 7 mm pockets but for patient KD, the 7 mm pocket decreased to 6 mm (Table 4).

When the paired T-Test was calculated, the change in 7 mm pockets was not significant (Table 3). The decrease in 8 mm pockets had a low significance with a p-value >0.10. However, the other changes were significant: 6 mm ($p>0.05$); 5 mm ($p>0.05$) and 4 mm ($p>0.01$).

To determine if there was a decrease in microorganisms after treatment, post treatment reports were compared with the baseline and all increases, decreases and lack of change were noted. To calculate the change in the Gram-negative and Gram-positive cocci and bacilli, for each of the areas that were sampled, (tongue base, tongue dorsum and the four quadrants) decreases were calculated as a percent of the total. For example, three out of the nine patients showed a decrease in Gram-positive cocci in the tongue base sample and this was recorded as one third of the test population showing a decrease or 33.33% (Table 5). Since the results could not be stated quantitatively, these results show a trend rather than a numerical decline.

Fusiforms, spirochetes, vibrio (strains of curved Gram-negative rods such as some Wollinella sp. or Capnocytophaga species[14]) and

Table 5: Percent Decrease in Microorganisms Post Treatment

PERCENT OF PATIENTS WITH POST TREATMENT RESULTS SHOWING DECREASE IN MICROORGANISMS							
Based on 9 patients	Tongue Base	Tongue Dorsum	Quad 1	Quad 2	Quad 3	Quad 4	Average
Gr+ Cocci	33.37%	44.44%	22.22%	11.11%	25.00%	44.44%	30.01%
Gr + Bacilli	66.67%	66.67%	55.56%	77.78%	50.00%	66.67%	63.89%
Gr - Cocci	44.44%	33.37%	33.33%	22.22%	37.50%	16.67%	31.26%
Gr- Bacilli	55.56%	66.67%	66.67%	88.89%	62.50%	55.55%	65.97%

Percent of post treatment results that show a decrease in the samples taken from the tongue base, tongue dorsum and quadrants. Quad= Quadrant; Gr+ =Gram-positive; Gr- = Gram negative.

yeast were present in some of the patients and these had the greatest decrease post treatment (Table 6). For those patients with amoeboid-like structures present in the biofilm, there was a decrease in numbers with the use of the antibiotic rinse (Table 7).

Discussion

The results provided strong evidence that an antibiotic mouth rinse containing metronidazole and nystatin is effective in the treatment of periodontitis as indicated by the tissue and biofilm changes. Gingival tissues were healthier, with fewer bleeding points and a decrease in periodontal pocket depth. These changes are brought about through decrease and in some cases elimination of many of the periodontal pathogens, such as oral spirochetes, implicated in periodontal disease[5] were not present in the second sample. Spirochetes have unique characteristics of locomotion that may enable them to penetrate and invade tissues[15] and have been widely recognized as important in the pathogenesis of periodontal disease.[15] Examinations of biofilm samples have been used in the detection of spirochetes which were then used for diagnosis and monitoring the need for periodontal treatment.[16]

The use of systemic metronidazole in the treatment of periodontal disease has been associated with significant reduction in the proportion and levels of spirochetes.[16] When used along with scaling and root planing, metronidazole has decreased periodontal pockets and significantly improved the outcome compared to scaling and root planing alone[17] and it is often the preferred antibiotic in the treatment of periodontal disease.[18] Further, it is well documented that metronidazole decreases periodontal pathogens, especially the fusospirochaetal complex.[19] It is also associated with rapid clinical improvement[19] and a gain in clinical attachment, indicating less need for surgery.[20] Once periodontal infection has been even partially resolved, it is important to place the patient on an individualized maintenance protocol with optimal biofilm control.[21] Combating periodontal infections is best accomplished when the choice of antibiotic is restricted to one that least is likely to develop resistance, such as metronidazole.[21,22] The use of this antibiotic as a rinse where it is not ingested further decreases the

Table 6: Pre and Post Treatment Comparisons

CHANGES IN MICROORGANISMS WITH ANTIBIOTIC RINSE TREATMENT						
	TB	TD	Q1	Q2	Q3	Q4
FUSIFORMS	7 (9)	5 (9)	8 (9)	7 (9)	7 (9)	9 (9)
eliminated	5	3	4	3	3	4
decreased	1	2	2	2	2	2
no change	1		1	1	1	3
increased			1		1	
SPIRO	4 (9)	4 (9)	5 (9)	5 (9)	3 (9)	4 (9)
eliminated	4	3	3	5	2	2
decreased			1		1	
no change			1			1
increased		1				1
VIBRIO	6 (9)	2 (9)	7 (9)	7 (9)	5 (9)	4 (9)
eliminated	6	2	5	7	1	2
decreased					2	
no change						1
increased			2		2	1
YEAST	5 (9)	4 (9)	2 (9)	3 (9)	3 (9)	4 (9)
eliminated	4	3	2	2	2	3
decreased						
no change						
increased	1	1		1	1	1

TB=tongue base; TD=tongue dorsum; Q=quadrant. Spiro=spirochetes.
Numbers inside the brackets are the patients assessed and the number beside the bracket is the number of microorganisms found in that group.

Table 7: Amoeboid Structures

NUMBER OF AMOEBOID STRUCTURES								
	Quadrant 1		Quadrant 2		Quadrant 3		Quadrant 4	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
GF	30	8	14	0	20	8	20	0
KG	0	0	10	0	0	0	2	0
MT	50	0	22	0	1	0	0	0

Changes in amoeboid-like structures post treatment.
Pre=Pretreatment; Post=Post treatment

Microbial diagnosis in the early-onset of disease is a recommended procedure to determine the status of disease

risk of systemic interaction and the development of microbial resistance.

The American Academy of Periodontology states that antibiotics may be prescribed on the basis of the clinical need for further treatment, the findings of microbiological testing and the medical status and current medications of the patient.[5] Diagnostic microbiology and the use of antibiotics should be considered as available tools in periodontal therapy as their combined use offers the clinician a high degree of efficacy and few or mild adverse effects. Microbial diagnosis in the early-onset of disease is a recommended procedure[23] to determine the status of disease. Microbial tests should then be applied to monitor the efficacy of mechanical treatment as well as antimicrobial chemotherapy and to determine the end-point of active treatment.[23] This system fits within the guidelines of the American Academy of Periodontology. The results indicate that a rinse system provides a beneficial adjunct in the treatment of periodontal disease. ■

REFERENCES

1. Position Paper: Diagnosis of Periodontal Diseases. J. Periodontol. 2003;74:1237-1247.
2. American Academy of Periodontology. Parameter on "refractory" periodontitis. J Periodontol. May 2000;71:859-860.
3. Position Paper:Systemic Antibiotics in Periodontics. J Periodontol. 2004;75:1553-1565.
4. Berezow AB and Darveau RP. Microbial shift and periodontitis. Periodontol 2000. 2011 February; 55(1):36-47.
5. Loesche WJ and Grossman NS. Periodontal disease as a specific, albeit chronic, infection: Diagnosis and treatment. Clinical Microbiology Reviews. 2001; 14(4): 727-752.
6. Lee HJ et al. Quantification of subgingival bacteria pathogens at different stages of periodontal disease. Curr. Microbiol published online April 2012. DOI10.1007/ 500284-012-0121-8.
7. Bhaijee F. and Bell D. Case Report – Entamoeba gingivalis in acute osteomyelitis of the mandible. Hindawi Publishing Corporation, Case Reports in Medicine. V 2011; Article ID 3570301- 3 pages.
8. Trim RD et al. Use of PCR to detect Entamoeba gingivalis in diseased gingival pockets and demonstrate its absence in healthy tissues. Parasitol Res published online 12 March 2011.
9. Kolenbrander PE et al. Bacterial interactions and successions during plaque development. Periodontology 2000. 2006; V42:47-79.
10. Baehni PC and Guggenheim B. Potential of Diagnostic Microbiology for Treatment and Prognosis of Dental Caries and Periodontal Diseases. Crit. Rev. Oral Biol. Med. 1996; 7(3): 259-277.
11. Mariotti A. and Monroe PJ. Pharmacologic management of periodontal disease using systemically administered agents. Advances in Periodontics, Part I Dental Clinics of North America, April 1998; 42(2):245-262.
12. Maestre JR et al. Odontogenic bacteria in periodontal disease and resistance patterns to common antibiotics used as treatment and prophylaxis in odontology in Spain. Rev Esp Quimioterap. March 2007;20(1):61-67.
13. Baltacioglu E et al. Analysis of clinical results of systemic antimicrobials combined with nonsurgical periodontal treatment for generalized aggressive periodontitis: A pilot study. J Can Dent Assoc 2011; 77:b97.
14. Tanner ACR et al. Wo Zinella gen. nov., WoZinella succinogenes (Vibriosuccinogenes Wolin et al.) comb. nov., and Description of Bacteroides gracilis sp. nov., Wolinella recta sp. nov.,Campylobacter concisus sp. nov., and Eikenella corrodens from humans with periodontal disease. International Journal of Systemic Bacteriology. 1981;3(4):432-445.
15. Hong L et al. Gene inactivation in the oral spirochete Treponema denticola; construction of an flgE mutant. Journal of Bacteriology. 1996; 178(12):3664-3667.
16. Loesche WJ et al. The utility of the BANA test for monitoring anaerobic infections due to Spirochetes (Treponema denticol) in periodontal disease. Microscopic J Dent Res 1990; 69(10):1696-1702.
17. Feres M. et al. Metronidazole alone or with amoxicillin as adjuncts to non-surgical treatment of chronic periodontitis: a 1-year double blinded, placebo-controlled, randomized clinical trial. J Clin Periodontol 2012; 00: 000–000. doi: 10.1111/jcpe.12004
18. Ghayoumi N. The use of Metronidazole in the treatment of periodontal diseases. J West Soc Periodontol. 2001;49(2):37-40.
19. Oral Microbiology 5th ed. Editors Marsh PD and Martin MV. Churchill Livingston Elsevier 2009.
20. Loesche et al. Metronidazole in Periodontitis: reduced need for surgery. J Clin Periodontol. 1992;19:103-112.
21. Slots J. Selection of antimicrobial agents in periodontal therapy. J Periodont Res 2002; 37:389-398.
22. Mombelli A and Samaranayake LP. Topical and systemic antibiotics in the management of periodontal disease. Int Dent J. 2004; 54(1):3-14.
23. Walter C et al. Critical assessment of microbiological diagnostics in periodontal disease with special focus on Porphyromonas gingivalis. Schweiz Monattsschr Zahnmed. 2005;5(5):415-424.