THE ANTIMICROBIAL TREATMENT OF PERIODONTAL DISEASE: CHANGING THE TREATMENT PARADIGM

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ABSTRACT: Over the last 100 years, methods of surgical periodontal treatment have enjoyed a history of success in improving oral health. The paradigm of care is based on the "non-specific plaque hypothesis"—that is, the overgrowth of bacterial plaques cause periodontal disease, and the suppression of this overgrowth reduces disease risk. The central feature of this approach to care is the removal of inflamed gingival tissue around the teeth to reduce periodontal pocket depth, thereby facilitating plaque removal by the dentist and by the patient at home. Over the last 30 years, with the recognition that periodontal disease(s) is caused by specific bacteria and that specific antimicrobial agents can reduce or eliminate the infection, a second paradigm has developed. This new paradigm, the "specific plaque hypothesis", focuses on reducing the specific bacteria that cause periodontal attachment loss. The contrast between the two paradigms can be succinctly stated as follows: The antimicrobial therapy reduces the cause, while the surgical therapy reduces the result of the periodontal infection. The specific plaque hypothesis has two important implications. First, with the increasing attention to evidence-based models for prevention, treatment, outcome assessment, and reimbursement of care, increasing attention and financial effort will be channeled into effective preventive and treatment methods. Second, the recent observations that periodontal infections increase the risk of specific systemic health problems, such as cardiovascular disease, argue for the prevention and elimination of these periodontal infections. This review highlights some of the evidence for the specific plaque hypothesis, and the questions that should be addressed if antimicrobial agents are to be used responsively and effectively.

Key words. Periodontal disease, antimicrobials, clinical trials, metronidazole, clinical outcomes, anaerobes, local delivery devices, compliance.

(I) *Introduction*

Both dental decay and periodontal disease are pathophysiological responses of the teeth and surrounding structures to the overgrowth of bacteria, in the form of dental plaque, on the dento-gingival surfaces. Almost all treatment and prevention techniques have used non-specific debridement procedures as the primary means of controlling these plaque accumulations. This non-specific approach to the management of dental infections (the Non-specific Plaque Hypothesis) has been universally applied to all individuals, even though it has been known for many years that a minority of individuals have the majority of the dental morbidity. The severity and magnitude of dental disease in these individuals imply that they are either uniquely susceptible to the bacterial overgrowth (genetic factor) or that the bacterial overgrowth contains certain uniquely odontopathic bacterial species (specific infection factor) (Loesche, 1976).

Since 1960, beginning with the demonstration that dental decay, and periodontal disease, were transmissible bacterial infections in animal models (Fitzgerald, 1968; Keyes, 1968), there has been evidence that most forms of human dental decay and periodontal disease are diagnosable and treatable infections. This translates to the possibility that only those individuals with a clinical infection, or at risk for a clinical infection, need to be treated. This would represent a new treatment paradigm which comes at a time in which clinical dentistry is undergoing great changes due to the decline in dental decay, the aging of the population, and challenges to the traditional reimbursement schedules.

The sudden and dramatic decline in dental decay in many countries in the last half of the 20th century has resulted in large numbers of individuals retaining their teeth into old age. This, combined with the increased longevity of these individuals, is giving rise to historic levels of dentate adults in these countries. Increasing age is a major and unmodifiable risk factor for periodontal disease (Grossi *et al.*, 1994), and thus it is likely that the

absolute numbers of individuals with periodontally diseased teeth will increase in the immediate future. This emerging periodontal problem provides an opportunity to apply the specific infection paradigm so as to focus antimicrobial treatment upon only those individuals whose plaques are "infected" with periodontopathogens and are therefore at risk for periodontal disease. This focus becomes very relevant if recent epidemiological studies showing a relationship between periodontal disease and cardiovascular disease can be substantiated (DeStefano *et al.*, 1993; Loesche, 1994; Mattilla *et al.*, 1995; Beck *et al.*, 1996; Loesche *et al.*, 1998a). If periodontal disease is a risk factor for cardiovascular disease, then it is a modifiable risk factor, and both the patient and the physician will want to see it controlled. Because antimicrobial agents appear to be cost-effective relative to surgical procedures, intervention studies to determine whether control of periodontal disease can prevent cardiovascular disease will probably include one or more antimicrobial agents.

It is against this background that the usage of antimicrobial agents in periodontal disease will be discussed. Antimicrobial agents are not magic bullets that can be used indiscriminantly to treat any infection, let alone a polymicrobial, chronic infection such as periodontal disease. It is premature to have a "bottom line" approach to treatment, if the infrastructure behind the usage of antimicrobial agents is not understood. A series of questions relative to patient selection, choice of drug, dosage and duration of treatment, as well as the clinical endpoint(s), needs to be answered unequivocally before the dental professional becomes an infectious disease specialist. In this review, we will draw mostly upon the double-blind studies in order to answer some of these questions that will apply to the usage of systemic and the newly developed locally delivered antimicrobial agents.

(II) *Whom to Treat?*

Most periodontal patients do not need antimicrobial treatment. Antimicrobial therapy should be used only when the clinical situation is serious enough to warrant this treatment and there is an appropriate diagnosis of an infection. The answer, then, as to whom to treat would be those patients who have multiple sites of inflammation associated with probing depths $\geq$ 5 mm, and in whom a periodontal infection can be diagnosed. These patients would most likely receive periodontal surgery and post-operative antimicrobial treatment, as judged by the responses to a questionnaire the American Academy of Periodontology sent to its membership in 1981 and in 1988 (Anonymous, 1989). In 1981, some 904 clinicians (67% of responders) and in 1988 some 1120 clinicians (83% of responders) reported that they use antibiotics following surgery. In 1981, the 904 clinicians reported using 454 different dosage regimens, and in 1988, the 1120 clinicians reported using 312 different regimens for agents mostly belonging to the penicillin, tetracycline, and erythromycin family of agents. These responses indicate that antibiotic usage is routine following surgery, but that there is no uniformity of opinion as to which agent and what dosage to use.

Another type of patient to treat with antimicrobials would be the refractory patient. About 10 to 20% of patients are non-responsive to debridement procedures and access surgery and are considered as refractory to treatment (Hirshfeld and Wassermann, 1978; McFall, 1982). A wide array of antimicrobial regimens is used to treat the refractory patient (Gordon *et al.*, 1990). Open studies have shown the combination of metronidazole and amoxicillin (van Winkelhoff *et al.*, 1992), clindamycin (Gordon *et al.*, 1990; Walker *et al.*, 1993), metronidazole (Loesche *et al.*, 1984; Lundstrom *et al.*, 1984; Gusberti *et al.*, 1988; Winkel *et al.*, 1997), augmentin (Magnusson *et al.*, 1994), doxycycline (Lundstrom *et al.*, 1984), tetracycline (Rams *et al.*, 1984; Haffajee *et al.*, 1995), and ornidazole (Mombelli *et al.*, 1989) to be effective in refractory periodontitis patients. But only doxycycline has been shown to be effective in a double-blind study (McCulloch *et al.*, 1990), and some patients who were non-responsive to the doxycycline improved when subsequently treated with metronidazole (Aitken *et al.*, 1992).

This choice of patient—namely, those who would require surgery in their treatment—provides a new treatment outcome which can be measured, *i.e.*, the reduced need for surgery (Loesche *et al.*, 1991, 1992a, 1996). This outcome measures a tangible benefit to the patient, if some or all of the surgical procedures can be avoided. If antimicrobial usage is restricted to the potential surgical patient, then the unwarranted usage of systemic and locally delivered antimicrobial agents in gingivitis and in moderate forms of periodontal disease would be discouraged. Some exceptions would exist, notably the treatment of necrotizing ulcerative gingivitis and periodontitis in HIV-positive patients, where metronidazole is often the agent of choice (Winkler *et al.*, 1989). Another would be acute necrotizing ulcerative gingivitis (ANUG), in which the effectiveness of metronidazole in anaerobic infections was discovered by Shinn in 1962, and subsequently established in a double-blind study (Duckworth *et al.*, 1966).

Patients who probably should not be treated with systemic antimicrobials include those diagnosed with localized juvenile periodontitis (LJP). This is a surprising recommendation, since it was these patients who were successfully treated with tetracyclines in open studies (Lindhe, 1982; Mandell *et al.*, 1986; Novak *et al.*, 1991). But a double-blind study in LJP patients found no benefits of

doxycycline beyond that obtained by scaling and root planing (Asikainen *et al.*, 1990), and other studies (Saxén and Asikainen, 1993; Gunsolley *et al.*, 1995) also concluded that LJP patients responded adequately to scaling and root planing and did not need any systemic antimicrobial agents.

(III) *What Agent to Use?*

Clinical symptoms do not reveal which antimicrobial agent to use, since this choice depends upon the types of bacteria that are responsible for the periodontal pathology. If one considers that the host inflammatory response is to the non-specific overgrowth of all bacterial types in the plaque, *i.e.*, the non-specific plaque hypothesis, then one would want to use antimicrobial agents that kill as many bacterial species as possible. If one considers that the host response is to a limited number of bacterial types, *i.e.*, the specific plaque hypothesis, then an agent that is active against the periodontopathic species is indicated.

(A) The Non-specific plaque hypothesis

It is estimated that there may be over 400 distinct species that can be found in the dental plaque, and when extensive efforts were made to classify the cultivable isolates, many were found to be previously undescribed species (Moore and Holdeman-Moore, 1994). In fact, most cultivable species were present in such low proportions that it was difficult to identify any single species as being uniquely associated with periodontal disease. This type of complexity supports the Non-specific Plaque Hypothesis' position, and if this be the case, then the flora would need to be suppressed either continuously or periodically. This can be best done by a treatment philosophy that relies upon mechanical debridement for bacterial control.

When this traditional debridement approach fails, as in the patients with refractory periodontitis, antimicrobial agents are often used to kill as many bacterial types as possible. This encourages the usage of broad-spectrum agents, or the combination of agents such as amoxicillin and metronidazole (van Winkelhoff *et al.*, 1992). Since the plaque flora would have to be suppressed either continuously or periodically, this approach could lead to the over-usage of these agents. Consider the following quote, taken from a report evaluating the ability of clindamycin to bring under control the deteriorating situation found in refractory patients: "During the year prior to entering the study, each patient had received antibiotics as part of the periodontal treatment. Tetracycline therapy ranged from one week's to one year's duration, with most patients receiving four or five administrations of 250 mg qid for 10 to 14 days. Every patient was also treated with at least one other antibiotic including penicillin V, ampicillin, augmentin, erythromycin, cephalexin and metronidazole" (Gordon *et al.*, 1990). In another report, nine refractory patients were treated with either penicillin, tetracycline, minocycline, or metronidazole (Vandekerckhove *et al.*, 1997). In still another study, 17 different antimicrobial regimens were used by 23 clinicians in the treatment of recurrent periodontitis (Levy *et al.*, 1993).

These reports indicate that the clinicians do not know which antimicrobial agent to use in their search for the "magic bullet" that will kill or suppress all plaque bacteria. This is because no agent can prevent or control "all" the 400 types of bacteria that can grow on tooth surfaces. This is the legacy of the non-specific plaque hypothesis, because without a targeted pathogen(s), it is very difficult to select the appropriate antimicrobial agent. But what would the scenario be if there actually were specific bacterial pathogens in periodontal disease?

(B) The specific plaque hypothesis

In the past 25 years, there have been over 100 studies which have compared the flora of disease-associated plaques with the flora found in plaques associated with periodontal health. This interest in the bacteriology of periodontal disease was generated by the association of A. *actinomycetemcomitans* with LJP (Newman and Socransky, 1977; Zambon, 1985). Previously, LJP was considered a degenerative condition known as periodontosis, and a diagnosis of periodontosis often resulted in extraction of the involved teeth. But with the recognition of LJP as an infection, treatments aimed at the reduction of A. *actinomycetemcomitans* in the plaques resulted in the retention of teeth that formerly were considered hopeless (Lindhe, 1982; Saxén and Asikainen, 1993). Thus, a new treatment paradigm was introduced, namely, that LJP was a treatable bacterial infection.

This led investigators to inquire as to whether other forms of periodontal disease could also be associated with specific bacterial types. The older literature identified anaerobic organisms such as spirochetes and black-pigmented Bacteroides species (now classified as *Porphyromonas* and *Prevotella* species) as putative periodontal pathogens (MacDonald *et al.*, 1962; Rosebury, 1962). The spirochetes were shown on microscopic examination of plaques samples to increase in both numbers and proportions as the clinical condition worsened (Listgarten and Helldén, 1978; Lindhe *et al.*, 1980; Loesche and Laughon, 1982, 1985; Riviere *et al.*, 1995). But with the identification of A. *actinomycetemcomitans* as a putative periodontal pathogen, emphasis shifted from anaerobes to this micro-aerophilic species. Selective media were developed that allowed it to be detected even when it was greatly outnumbered by other plaque species (Slots, 1982; Mandell *et al.*, 1987). As a result, it was implicated, on the basis of association-type studies,

TABLE 1

Bacterial Species Suspected to be Periodontopathogens

Species	Clinical Entity*	Oxygen Sensitivity
Porphyromonas (Bacteroides) gingivalis	AP, EOP, RP	Anaerobic
Bacteroides forsythus	AP, EOP, RP	Anaerobic
Treponema denticola	AP, EOP, RP	Anaerobic
Prevotella intermedia	AP, ANUG	Anaerobic
Fusobacterium nucleatum	AP	Anaerobic
Eubacteria nodatum	AP	Anaerobic
Selenomonas noxia	AP	Anaerobic
Porphyromonas (Bacteroides) gracilis	AP	Anaerobic
PROS Spirochete (*Treponema vincentii*)	AP, ANUG	Anaerobic
Peptostreptococcus micros	AP, RP	Anaerobic
Eubacterium sp.	AP	Anaerobic
Selenomonas sp.	AP	Anaerobic
Streptococcus intermedius	AP, RP	Anaerobic
Actinobacillus actinomycetemcomitans	LJP, EOP? RP?	Micro-aerophilic
Wolinella (Campylobacter) recta	AP	Micro-aerophilic
Eikenella corrodens	AP?	Micro-aerophilic

*AP = adult periodontitis, EOP = early-onset periodontitis, ANUG = acute necrotizing ulcerative gingivitis, LJP = localized juvenile periodontitis, RP = refractory periodontitis, ? = evidence is equivocal.

as a putative periodontal pathogen in refractory periodontitis, early-onset periodontitis, and in the rapidly deteriorating lesion (Tanner *et al.*, 1979; Dzink *et al.*, 1988; van Winkelhoff *et al.*, 1989; Slots *et al.*, 1990a; Haffajee and Socransky, 1994). Its persistence in plaque and presence within the gingival tissue were used to design antimicrobial regimens to eradicate it from both the plaque and the gingiva (Slots and Rosling, 1983; Mandell and Socransky, 1988; Goene *et al.*, 1990; Christersson and Zambon, 1993; van Winkelhoff *et al.*, 1992; Goodson, 1994).

These reports indicated that both A. *actinomycetemcomitans* and anaerobes can be associated with periodontal disease. Thus, a comparison of the relative importance of A. *actinomycetemcomitans* and anaerobes would be a necessary starting point to decide which antimicrobial agent to use in periodontal disease.

(C) Is periodontal disease an anaerobic or micro-aerophilic infection?

This question can be answered by studies which monitor the majority of the plaque bacterial types. We reported on both the prominent cultivable flora as well as the microscopic counts of over 400 plaque samples taken from 120 patients, including periodontal patients treated successfully, and untreated patients with early-onset periodontitis, adult periodontitis, and LJP (Loesche *et al.*, 1985). Only spirochetes were significantly elevated, both in absolute numbers and in proportions, in plaques removed from untreated patients compared with plaques in the treated patients. *Porphyromonas gingivalis* was significantly increased in the patients with early-onset periodontitis. Facultative species such as S. *sanguis* and A. *viscosus* were significantly elevated in the treated patients. A. *actinomycetemcomitans* could not be detected, even in the LJP patients. Subsequently, we examined over 200 plaques removed from teeth scheduled for periodontal surgery and, using DNA probes, polyclonal antibodies, and cultural methods, could detect A. *actinomycetemcomitans* in about 20 to 50% of the plaques (Loesche *et al.*, 1992b). However, P. *gingivalis*, *Bacteroides forsythus*, *Treponema denticola*, and spirochetes were present in 80 to 100% of the plaques, indicating that these anaerobes were more prevalent and dominant relative to A. *actinomycetemcomitans*.

Other investigators have reported that A. *actinomycetemcomitans* could not be associated with periodontal disease. Moore and Holdeman-Moore (1994), using culture methods, identified *Fusobacterium nucleatum* and spirochetes with periodontal disease, but not A. *actinomycetemcomitans*. In a cross-sectional study involving over 1300 subjects, Grossi *et al.* (1994, 1995) significantly associated B. *forsythus* and P. *gingivalis*, but not A. *actinomycetemcomitans*, with either attachment loss or alveolar bone loss. Socransky and colleagues (1998), using DNA probes, examined over 13,000 plaques removed from 185 individuals for the presence of 40 bacterial species. Only T. *denticola*, P. *gingivalis*, and B. *forsythus* could be statistically associated with increasing pocket depth and bleeding on probing. The same three species plus *Campylobacter* (*Wolinella*) *recta*, of 18 species monitored with DNA probes, were associated with periodontal disease and progressive disease in 148 adult Chinese who had never received any type of periodontal treatment (Papapanou *et al.*, 1997).

At least 12 other groups have found anaerobic species to be more prevalent than A. *actinomycetemcomitans* in plaques taken from diseased periodontal sites. Ashimoto *et al.* (1996), using a PCR technique, found the prevalence of anaerobes such as B. *forsythus* to increase 10.7-fold, of P. *gingivalis* to increase five-fold, and T. *denticola* to increase 3.4-fold, when plaques from diseased sites in adults were compared with plaques removed from sites of gingivitis in children. Micro-aerophilic species showed minimal changes, *i.e.*, A. *actinomycetemcomitans* increased 2.1-fold, C. *recta* showed no increase

and *Eikenella corrodens* increased 1.2-fold. Lowenguth *et al.* (1995), using DNA probes, found that A. *actinomycetemcomitans* was present in 5.5% of 219 sites that were treated with a 25% tetracycline fiber, whereas F. *nucleatum* was present in 70.8%, P. *gingivalis* in 43%, and *Prevotella intermedia* in 63.5% of the sites. In a clinical trial involving azithromycin, A. *actinomycetemcomitans* was found in six of 46 subjects, P. *gingivalis* was present in nine of 44, P. *intermedia* in 21 of 44, any black-pigmented anaerobes in 39 of 44, and spirochetes in 40 of 44 subjects (Sefton *et al.*, 1996). A. *actinomycetemcomitans* could be detected in 16% of 196 subgingival plaque samples removed from sites of refractory or recurrent periodontitis, whereas B. *forsythus* was detected in 84% of the samples, spirochetes in 83%, *Fusobacterium* species in 68%, and P. *gingivalis* in 63% (Listgarten *et al.*, 1993).

Umeda *et al.* (1996) examined plaques removed from pockets showing clinical symptoms of pain and suppuration and found spirochetes to account for 27% of the microscopic count, and P. *gingivalis* and B. *forsythus* to account for 26% and 11%, respectively, of the cultivable count. A. *actinomycetemcomitans* accounted for only 0.3% of the cultivable count and tended to increase as a result of treatment. Kamma *et al.* (1994) found F. *nucleatum* and P. *gingivalis* to be prevalent in 90% of 73 plaques removed from sites with depths > 6 mm in refractory patients. A. *actinomycetemcomitans* was found in only two of the 10 patients and in 11% of the sites. B. *forsythus* was present in 53% of the sites and, together with P. *gingivalis*, accounted for 23.6% and 26.7% of the cultivable flora. The indirect fluorescent antibody technique is more likely than other techniques to detect A. *actinomycetemcomitans* in plaque samples (Loesche *et al.*, 1992b). Christersson *et al.* (1992), using an indirect fluorescent antibody technique, examined subgingival plaques taken from the mesial surfaces of all teeth in 12 patients with adult periodontitis for P. *gingivalis*, A. *actinomycetemcomitans*, B. *forsythus*, and P. *intermedia*. The three anaerobes were present in 44 to 54% of the plaques, while A. *actinomycetemcomitans* was found in only 11% of the plaques. Still others, while monitoring the effect of locally delivered antimicrobials upon the plaque flora, have noted that anaerobes are both more prevalent and more numerous than A. *actinomycetemcomitans* (Goodson *et al.*, 1991; Jones *et al.*, 1994; Timmerman *et al.*, 1996; Bollen *et al.*, 1998).

These studies, from many laboratories involving large numbers of samples and using diverse methods, indicate that anaerobes, rather than A. *actinomycetemcomitans*, are more likely to be present in, or dominate, plaques associated with disease. While B. *forsythus*, P. *gingivalis*, and T. *denticola* are the most commonly found anaerobes, others, such as *Peptostreptococcus micros*, are also found (Table 1).

(D) Diagnosis of an anaerobic infection

These bacteriological findings indicate that most, if not all, forms of periodontal disease are anaerobic infections due to the overgrowth of a finite number of mostly Gram-negative bacteria. But how does a clinician diagnose this infection? The phase contrast microscope combined with a video camera can be used at chairside to record the architecture of the plaque microbial community, the motility of its members, and the number of white blood cells (Keyes *et al.*, 1978). The darkfield microscope (Listgarten and Helldén, 1978) can be used to enumerate the numbers of spirochetes in plaques removed from the most diseased site in each quadrant. Because spirochetes can be found in most individuals (Loesche and Laughon, 1982), we require that each of 3 or 4 of the 4 sampled plaques have 20% or more spirochetes before we diagnose an anaerobic infection.

The BANA test, a five- to 10-minute chairside test based on the ability of the 4 plaque samples to hydrolyze the synthetic trypsin substrate, N-benzoyl-DL-arginine-2-naphthylamide (BANA), can be used to diagnose an anaerobic infection (Loesche *et al.*, 1992c, 1997). P. *gingivalis*, B. *forsythus*, and T. *denticola* each possess an enzyme(s) capable of hydrolyzing BANA, whereas 55 other cultivable plaque species do not (Loesche *et al.*, 1990). As noted previously, these BANA-positive organisms have been significantly associated with periodontal disease in both American (Grossi *et al.*, 1994; Ashimoto *et al.*, 1996; Socransky *et al.*, 1998) and Chinese populations (Papapanou *et al.*, 1997). While the BANA test does not discriminate as to which of the three species are present, this may not be necessary, since these species tend to co-exist in the same plaques (Haffajee *et al.*, 1998; Socransky *et al.*, 1998), and all are anaerobes. Thus, the BANA test is essentially used to diagnose an anaerobic infection.

The plaque samples could be sent to reference laboratories for cultural, immunological, or DNA probe analysis (Listgarten, 1992; Rams *et al.*, 1992; Zambon, 1997). Cultural analysis would allow for the growth of only those anaerobes that survive the transport process. However, it would permit investigators to determine whether, among those that grow, the suspected periodontopathogen is resistant to an antibiotic such as doxycycline. Such testing may not be necessary for metronidazole, since all anaerobes are uniquely sensitive to this agent (Tally *et al.*, 1978), and resistance to metronidazole in a clinical setting is extremely rare (Walker *et al.*, 1985; Garcia-Rodriguez *et al.*, 1995).

(E) Agents active against anaerobes

Doxycycline, clindamycin, members of the penicillin family, and metronidazole and its analogues are active

TABLE 2

Double-blind Studies of Systemic Metronidazole in Periodontal Disease Listed According to Total Dosage Dispensed

Study	Daily Dosage	Length of Treatment	Total Dosage	Dosage Relative to Debridement	Other TX	Outcome
Duckworth *et al.*, 1966	200 mg tid	2 days	1.2 g	—	—	• Ulcer in ANUG
Sterry *et al.*, 1985	200 mg tid	7 days	4.2 g	after	curettage	No effect - Cross-over study
Mahmood and Dolby, 1987	200 mg tid	7 days	4.2 g	after	surgery	No effect - Cross-over study
Watts *et al.*, 1986	200 mg tid	7 days	4.2 g	none	—	• Bleeding
Clark *et al.*, 1983	250 mg tid	7 days	5.25 g	after	—	≈ Attachment level
Loesche *et al.*, 1984	250 mg tid	7 days	5.25 g	at beginning	—	≈ Attachment level
Loesche *et al.*, 1991	250 mg tid	7 days	5.25 g	at beginning	—	• Need for surgery
Loesche *et al.*, 1992a	250 mg tid	7 days	5.25 g	after	—	• Need for surgery
Joyston-Bechal *et al.*, 1984	200 mg qid repeated after 4 wks	5 days x2	8 g	after	Chx gel	• Probing depth
Söder *et al.*, 1990	400 mg tid	7 days	8.5 g	after	—	• Probing depth
Loesche *et al.*, 1996	500 mg bid	14 days	14 g	after	—	• Need for surgery
Lindhe *et al.*, 1983	200 mg qid repeated 2 times at 8-wk intervals	14 days • x3	33.6 g	after	—	≈ Attachment level

• Decrease in measured outcome parameter.

≈ Increase in measured outcome parameter.

against anaerobes. Other agents such as the quinolones are also active, but because of their importance in medical infections and the development of cross-resistance between members of the quinolone family (Andriole, 1998), they should not be used.

Of these agents, metronidazole would be the first choice because it is specific for anaerobes and would not affect the facultative flora which appears to be associated with health (Loesche *et al.*, 1985; Socransky *et al.*, 1998). Metronidazole has also been the most extensively evaluated of these agents in periodontal disease (Table 2). There are eight double-blind studies which have shown it to be clinically effective in periodontal disease (Duckworth *et al.*, 1966; Lindhe *et al.*, 1983; Joyston-Bechal *et al.*, 1984; Loesche *et al.*, 1984, 1991, 1992a, 1996; Söder *et al.*, 1990); two double-blind studies which showed a tendency for metronidazole to be effective, but which lacked enough subjects to show statistical significance (Clark *et al.*, 1983; Watts *et al.*, 1986); and two double-blind studies, each using a cross-over design, which showed it to have no value as a prophylactic agent when used in conjunction with access surgery (Mahmood and Dolby, 1987) or curettage (Sterry *et al.*, 1985).

Doxycycline has a broader spectrum of antimicrobial activity than metronidazole, being active against some micro-aerophilic organisms. Two double-blind studies (McCulloch *et al.*, 1990; Loesche *et al.*, 1996) have shown it to be clinically effective in adult forms of periodontal disease, but not in LJP patients (Asikainen *et al.*, 1990). Tetracycline would not be an acceptable choice, since approximately 12% of the subgingival plaque flora are resistant to tetracycline, and of these, all the species containing the Tet Q gene were Gram-negative anaerobic bacteria, including all *Prevotella* and *Bacteroides* isolates (Lacroix and Walker, 1995). There are few well-controlled studies that have shown its efficacy in periodontal disease. A meta-analysis of 42 studies involving the usage of tetracycline in the period from 1965 to 1988 could find no evidence of efficacy, primarily because of the absence of properly controlled studies (Hayes *et al.*, 1992). A recent double-blind study involving patients with both LJP and early-onset periodontitis showed a short-term, *i.e.*, three-month, benefit in pocket reduction and attachment gain in the tetracycline-treated group which disappeared after the teeth were treated by periodontal surgery (Palmer *et al.*, 1996).

The penicillin family, which would include ampicillin, amoxicillin, and augmentin, has been shown, in

open studies, to be effective in refractory periodontitis, either when given alone (Walker *et al.*, 1993) or when combined with metronidazole (van Winkelhoff *et al.*, 1992). But the efficacy of these agents for serious medical infections is threatened by the emergence of beta-lactamase-positive species, so it would seem inappropriate to use these agents in periodontal disease when alternate antimicrobials are available. Several plaque species produce beta-lactamases (Legg and Wilson, 1990; van Winkelhoff *et al.*, 1997), and this enzyme is detectable in gingival crevicular fluid (GCF) (Gordon and Walker, 1993), suggesting the presence of penicillin-resistant organisms. Periodontal abscesses have been reported when patients with periodontal disease were treated with penicillin for medical reasons (Helovuo *et al.*, 1993; Topoll *et al.*, 1990).

The combination of metronidazole and amoxicillin was chosen for some clinical studies because A. *actinomycetemcomitans* was sensitive to this combination of agents *in vitro* (Pavicic *et al.*, 1992). This combination will eliminate A. *actinomycetemcomitans* from plaque samples, will reduce probing depths, and will increase attachment in patients diagnosed as having LJP, refractory periodontitis, or generalized periodontitis (van Winkelhoff *et al.*, 1992). However, this was an open study in which only before-and-after comparisons were made, and there were no controls. Given the proven ability of metronidazole, in double-blind studies, both to lower probing depths and to increase attachment levels (Lindhe *et al.*, 1983; Joyston-Bechal *et al.*, 1984; Söder *et al.*, 1990; Loesche *et al.*, 1992a; Loesche and Giordano, 1994), it is likely that most of the improvements noted could be attributed to the metronidazole. Metronidazole alone has been shown to be effective in reducing the levels of A. *actinomycetemcomitans* in plaques removed from LJP patients (Saxén and Asikainen, 1993). It would seem premature to recommend the additional usage of amoxicillin without evidence that the combination of amoxicillin and metronidazole is clinically superior to either metronidazole alone, or to amoxicillin alone.

An industry-formulated combination of 125 mg metronidazole and 750,000 IU of spiramycin in a drug known as Rodogyl has been evaluated in a double-blind clinical trial (Quee *et al.*, 1987). This formulation was based on *in vitro* data which showed this combination to require one-tenth the spiramycin and one-thirtieth the metronidazole to inhibit anaerobic bacteria than when they were used individually. In the double-blind study, 50 adult periodontitis patients were subjected to scaling and root planing and were given three tablets of Rodogyl or placebo *per* day for 14 days. Compared with the placebo group, the Rodogyl group exhibited a significant average gain of 0.67 mm in attachment levels and almost complete suppression of spirochetes in the plaque for up to 6 months after treatment.

Azithromycin, a macrolide with bacteriostatic activity against oral anaerobes *in vitro*, was shown, in a double-blind study, to reduce probing depths significantly in those sites initially > 6 mm, when compared with a placebo control (Sefton *et al.*, 1996). Azithromycin is concentrated in polymorphonuclear and mononuclear cells (Calia and Oldach, 1998), and since many of these cells exit into the pocket (Skapski and Lehner, 1976), they would, after lysis, release elevated levels of this agent in the vicinity of plaque anaerobes. Azithromycin has been able to reduce secondary medical outcomes in patients with cardiovascular disease (Gupta *et al.*, 1997). This agent was chosen because *Chlamydia pneumoniae*, the bacterial species most frequently associated with cardiovascular disease (Matilla *et al.*, 1998), can live within macrophages, and thus would be exposed to the high concentrations of azithromycin found therein. But azithromycin could improve the periodontal health of the patient, so that some of the beneficial results obtained in the Gupta *et al.* study could reflect a periodontal effect.

Clindamycin might be a suitable choice because of its activity against anaerobes, but no double-blind studies exist to show its clinical efficacy in periodontal disease. An open study with before-and-after measurements showed it to be effective in 30 refractory patients who had been previously treated with various combinations of systemic agents (Gordon *et al.*, 1990).

It has been suggested that a sequence of antimicrobial agents may be more effective than either agent used alone or in combination. This possibility was based on the finding that refractory patients, who did not respond to doxycycline, were more responsive to a subsequent treatment with metronidazole than were the former placebo patients when they were also treated with metronidazole (Aitken *et al.*, 1992). In our 4th double-blind study (Loesche *et al.*, 1996), patients who still had > 6 teeth in need of periodontal surgery or extraction after the first round of systemic agents were re-treated with the antimicrobial opposite that with which they had initially been treated. Both the metronidazole/doxycycline sequence patients and the doxycycline/metronidazole sequence patients responded equally well to the second treatment. This suggests that the additional improvements noted by Aitken *et al.* were more a function of dosage, *i.e.*, being treated twice, than of any uniqueness associated with the sequence.

(F) Safety considerations

All medications have potential side-effects, such as the ability to evoke an allergic reaction. The clinician needs to verify, prior to treatment, that no such problem is known to exist, and to stop treatment if complaints consistent with an allergic reaction are reported by the

patient. There are specific concerns related to each of the recommended agents.

(1) Metronidazole

(a) Bacterial resistance

Since 1958, metronidazole has been the treatment of choice for *Trichomonas vaginitis* and other protozoa infections. Its usage for anaerobic infections stems from the observation by Shinn (1962) of its efficacy in ANUG. Testing of plaque anaerobes for metronidazole resistance has not disclosed any pattern of increased resistance over time (Listgarten *et al.*, 1993). The emergence of resistant anaerobes among medical isolates is rare (Garcia-Rodriguez *et al.*, 1995). The exposure of reference strains and recent plaque isolates on serial passage to sub-inhibitory levels of metronidazole resulted in the development of a low level of resistance that was deemed not to be of clinical significance if metronidazole was not used repeatedly (Larsen and Fiehn, 1997). We have not observed the development of resistant black-pigmented anaerobes in our studies, but A. *actinomycetemcomitans* strains resistant to 25 μg/mL have been reported by van Winkelhoff *et al.* (1992) in patients in whom A. *actinomycetemcomitans* could not be eliminated. Thus, the emergence of clinical resistance of microaerophilic species to metronidazole is a possibility. This potential concern needs to be balanced by a risk-to-benefit ratio, and this is why we have restricted our usage of metronidazole to only those patients in whom an anaerobic infection can be diagnosed.

(b) Mutagenicity

Ames *et al.* (1987) developed a bacterial assay to screen chemicals for mutagenicity, to serve as a surrogate for the animal studies which had been used to screen these chemicals for potential carcinogenicity. The assay involved exposing a mutated form of *Salmonella typhimurium* to the chemical in question, and then looking for growth of mutants. The Ames test was positive for known mutagens and was then used to determine the potential mutagenicity of previously untested chemicals. Antimicrobial agents like penicillin were lethal for the test organism, and therefore their mutagenicity could not be assessed. However, because metronidazole would not kill S. *typhimurium*, a facultative organism, metronidazole was found to be mutagenic in the Ames test.

Subsequent studies in mice showed that extremely high dosages of metronidazole (625 mg/kg of animal weight) could be associated with cancer in female animals (Rustia and Shubik, 1972); the comparable daily dose in a 150-pound human would be 43 g (Roe, 1983). (This human would have to take 86 tablets of 500 mg metronidazole a day for a lifetime to be compliant!) The fact that metronidazole is used primarily for treating *Trichomonas* vaginal infections caused the National Organization of Women to petition the FDA in 1972 to suspend the sales of metronidazole. The FDA concluded that metronidazole should remain on the market because of the absence of any adverse effects in humans, and because other animal studies using very high dosages could not duplicate the above findings (Roe, 1983). They did include a warning label on the pocket insert in each box that high dosages of metronidazole can cause tumors in certain small rodents.

There have been 32 studies seeking to find any association between metronidazole, when used to treat *Trichomonas* infections in pregnant women, and birth defects. Burtin *et al.* (1995) performed a meta-analysis on these studies and concluded that metronidazole does not appear to be associated with an increased teratogenic risk. Periodontal disease has been suggested as a possible risk factor for causing the premature birth of low-birth-weight babies (Offenbacher *et al.*, 1996). If the women were diagnosed with a vaginal infection and treated with metronidazole, the prevalence of premature births was decreased. It is possible that some of this clinical efficacy of metronidazole in preventing pre-term births could have involved an improvement in periodontal health. The fact that metronidazole can be considered as a treatment for pregnant women should allay any concerns about the safety of this agent.

Eventually, the Ames test was used to screen presumably harmless chemicals such as various foods, and when they were found to be mutagenic, the usefulness of the Ames test as a screening assay became questionable, *i.e.*, there were too many false-positive results (Ames, 1986; Ames *et al.*, 1987). In fact, if the extrapolations of a positive Ames test to cancer were correct, the prevalence of environmental mutagens is so high that the human population should long ago have been wiped out by cancer.

(c) Anabuse effects

Metronidazole can combine with alcohol to cause acute nausea in some individuals. Accordingly, a label warning users not to drink alcoholic beverages is appended to the metronidazole bottle.

(2) Doxycycline

(a) Bacterial resistance

Bacterial resistance to the tetracycline family of antimicrobial agents, including doxycycline, is common among the oral flora. About 2 to 6% of the subgingival flora and 3 to 12% of the flora on the tonsils in periodontally healthy subjects, who were not taking doxycycline, were resistant to doxycycline (Fiehn and

Westergaard, 1990). When doxycycline was given for three weeks to periodontally diseased patients, the number of doxycycline-resistant bacteria increased 10- to 20-fold in the plaque and on the tonsils, respectively. These values returned to pretreatment levels within six months. In another study (Rams *et al.*, 1990), a three-week course of doxycycline resulted in more than a 10-fold increase in subgingival levels of medically important pathogens such as staphylococci (11 of 21 patients), *Escherichia coli* (one patient), *Enterobacter aerogenes* (two patients), and *Candida albicans* (two patients). These findings are of concern, since these organisms could contribute to a persistent type of refractory periodontitis that would be resistant to most antimicrobial agents (Slots *et al.*, 1990b).

Tetracycline resistance is a major concern, because the resistance gene is located on plasmids near the insertion sites. This is conducive to the development of multiple drug resistance if the plasmid acquires other genetic information for drug resistance at this insertion site. The tetracycline molecule can be modified, as in the case of doxycycline, to inhibit certain tetracycline-resistant forms of streptococci. However, the oral streptococci still remain among the most likely species to become resistant to doxycycline (Fiehn and Westergaard, 1990; Olsvik and Tenover, 1993). But since these organisms are not periodontopathic, and the period of increased resistance is transient, no one has yet attributed any clinical concerns to this event.

(b) Other complications

The tetracyclines, like many antimicrobials, can cause diarrhea, but also have certain adverse reactions that are unique to them, such as a photosensitivity to sunlight. This response is less likely to occur with doxycycline, but patients should be advised to avoid sunbathing while taking this agent. Another complication that would be of concern to women of childbearing age is the ability of tetracyclines to act indirectly with oral contraceptives in the intestinal tract, thereby decreasing their absorption and possibly leading to an unwanted pregnancy (Barnett, 1985).

(3) Clindamycin

Clindamycin is bactericidal for aerobic Gram-positive cocci and most anaerobic bacterial species (Oldach and Calia, 1998). This spectrum of activity has made it an attractive agent for use in anaerobic pelvic, abdominal, and pulmonary infections. Its main disadvantage is the high prevalence of diarrhea, *i.e.*, from 2% to 30% (average, 8%), that would make this an unacceptable choice, considering the availability of metronidazole and doxycycline. More ominous is the possibility that the diarrhea could become a life-threatening super-infection due to *Clostridium difficile*. This anaerobe is often a normal inhabitant of the large intestine and, because of its resistance to clindamycin, is able to overgrow and cause severe colitis. The incidence of significant colitis due to clindamycin ranges from 0.1% to 10% (Oldach and Calia, 1998). One of 30 refractory periodontal patients (3%) treated with clindamycin developed colitis (Gordon *et al.*, 1990).

(4) Local delivery devices

(a) Lower total body dosages

The concern over the possibility of some individuals experiencing an adverse reaction to systemic agents and the easy access of the dento-gingival surfaces have encouraged the development of vehicles to release antimicrobial agents directly into the periodontal pocket. This is a very positive development from many perspectives, not the least of which is safety due to lower total body doses of the agents.

For example, the average tetracycline content of a single 25% tetracycline fiber (Actisite®) placed in a pocket is about 8 mg/tooth (Goodson, 1994). If 12 teeth are treated for 10 days, the total tetracycline fiber-dosage is about 96 mg. But only 25% of this dosage is released during the fiber's stay in the pocket, giving a total body dosage of 24 mg. The comparable systemic dosage would be 1 gram for 14 days, or 14,000 mg, so that the use of the fibers reduces the tetracycline dosage by more than 99.5%. Similar reductions in dosage would be obtained with the 25% metronidazole gel (Elyzol®), which delivers about 3 mg of agent *per* tooth. However, this regimen needs to be repeated, and since the gel is biodegradable, all of the metronidazole reaches the body (Stoltze, 1995), so that the total body dosage for the same 12 teeth would be 72 mg. But this represents a 99% reduction in whole-body dosages compared with metronidazole administered systemically, 500 mg twice a day for one week.

It is difficult to imagine an anabuse effect from the local deposition of a 25% metronidazole gel (Ainamo *et al.*, 1992), or from the placement of a thin film containing metronidazole in the periodontal pocket (Loesche *et al.*, 1996), even if multiple pockets are treated simultaneously. Nor can one imagine a tetracycline-impregnated fiber (Goodson, 1994) or doxycycline- or minocycline-containing gels causing diarrhea (Graca *et al.*, 1997; Polson *et al.*, 1997b). Moreover, an agent like chlorhexidine, which is too toxic to be taken systemically, can now be incorporated into a thin film which is deposited in a pocket (Soskolne *et al.*, 1997). Thus, when high concentrations of an agent are incorporated into a vehicle that delivers the agent directly to the periodontal pocket, many of the side-effects associated with whole-body dosing, as would occur with systemic agents, should be avoided.

TABLE 3

Double-blind Studies of Systemic Antibiotics in Periodontal Disease

Agent	Study	Daily Dosage	Length of Treatment	Total Dosage	Dosage Relative to Debridement	Outcome
Doxycycline	McCulloch *et al.*, 1990	100 mg	21 days	2.2 g	After	• Recurrence
	Asikainen *et al.*, 1990	100 mg	14 days	1.4 g	After	LJP - no effect
	Loesche *et al.*, 1996	100 mg	14 days	1.4 g	After	• Need for surgery
Tetracycline	Rams *et al.*, 1984	250 mg qid	14 days	14 g	After	
	Palmer *et al.*, 1996	250 mg qid	14 days	14 g	After	≈ Attachment level • Need for surgery
	Al-Joburi[a] *et al.*, 1989	250 mg qid	14 days	14 g	After	No effect
Rodogyl	Quee *et al.*, 1987		14 days		During	≈ Attachment level
metronidazole		750 mg		10.5 g		
spiramycin		4,500,000 IU		63 x 10^6 IU		
Azithromycin	Sefton *et al.*, 1996	500 mg	3 days	1.5 g	After	• Probing depth
Spiramycin	Al-Joburi[a] *et al.*, 1989	3,000,000 IU	14 days	42 x 10^6 IU	After	No effect
Combination	Lopez & Gamonal, 1998		7 days		No debridement	
metronidazole		250 mg tid		5.25 g		≈ Attachment level
amoxicillin		500 mg tid		10.5 g		• Probing depth

• Significant decrease in measured parameter.

≈ Significant increase in measured parameter.

[a] Same study.

(b) Bacterial resistance

The concerns related to bacterial resistance are reduced with the local delivery vehicles, because, as a result of dilution effects, there would be almost no exposure of the intestinal flora to an agent such as tetracycline and doxycycline. However, this does not mean that the emergence of resistant organisms will not occur in the oral cavity, since some members of the oral flora will be exposed to lower concentrations of the agent in the area away from the treatment site. In one study (Larsen, 1991), the level of doxycycline-resistant bacteria was 1% in the plaque, on the tongue, and on the tonsils prior to treatment. After treatment with locally delivered doxycycline, the percentages of resistant bacteria increased to 22% and 35%, respectively, on the tongue and tonsils, and then declined to pre-treatment levels by week 13. This transient increase was due primarily to resistant Gram-positive cocci and was without clinical significance.

(IV) *What Dosage to Use?*

(A) Systemic agents

Only a few dosage schedules are approved by the FDA. However, the creativity of the dental community in devising dosages is not to be underestimated, judging from the responses to the American Academy of Periodontology questionnaire cited earlier, where over 300 different antibiotic regimens were used following periodontal surgery (Anonymous, 1989). Even among the research investigations, it is difficult to compare between studies because of the different dosages used. If we restrict ourselves to the 12 double-blind studies involving metronidazole, most of the factors which confound comparisons between investigations become evident (Table 2).

(1) Metronidazole

The dosages of metronidazole ranged from 1.2 g (Duckworth *et al.*, 1966) to 33.6 g (Lindhe *et al.*, 1983), and the length of treatment varied from 2 days to 42 days. In most studies, the metronidazole was combined with mechanical debridement, but in one study there was no debridement (Watts *et al.*, 1986). Sometimes the taking of the metronidazole occurred at the beginning of debridement and other times after debridement had been completed. There would be fewer bacteria in the pocket in the latter situation, and this could improve the outcome because of a lower microbe-to-agent ratio (Loesche and Giordano, 1994) (Table 4). In two studies, the subjects were re-treated with metronidazole, thereby increasing the overall dosage (Lindhe *et al.*, 1983; Joyston-Bechal *et al.*, 1984). In one study, a chlorhexidine gel was combined with the metronidazole (Joyston-Bechal *et al.*, 1984).

These variables make comparisons between and

among studies difficult, but despite this, all but two showed a beneficial effect of metronidazole on the measured clinical outcomes. One failure involving a cross-over design showed no benefit of metronidazole when used following surgery (Mahmood and Dolby, 1987), and since this prophylactic indication is not of value for any antimicrobial agent (Pack and Haber, 1983; Tseng *et al.*, 1993), this outcome was to be expected. The other failure also involved a cross-over design in which both groups received metronidazole separated by a 10-week interval (Sterry *et al.*, 1985). Since the effect of metronidazole can last many months (Loesche *et al.*, 1991, 1992a), it is possible that, in both studies, the wash-out period between the different treatments was so short that all participants had benefited from the metronidazole.

The multiple dosage regimens make difficult the transference of the results to clinical practice. But a pattern is obvious: Metronidazole is best given for one week after debridement of the tooth surfaces. The total dosages should be at least 5.25 g, *i.e.*, 250 mg three times a day for one week, but as will be discussed subsequently, a twice-a-day (bid) regimen will improve compliance.

(2) Other agents

The dosages of doxycycline and tetracycline have been consistent between and among studies (Table 3). A two-week treatment of doxycycline was without effect in LJP patients (Asikainen *et al.*, 1990) but was effective in a study involving adults who needed periodontal surgery and were diagnosed with an anaerobic infection (Loesche *et al.*, 1996). A three-week treatment was associated with a significant improvement in refractory patients (McCulloch *et al.*, 1990). The results with tetracycline were equivocal.

Rodogyl®, the spiramycin-metronidazole combination tablet, was given as 3 tablets twice daily for two weeks in adult patients (Quee *et al.*, 1987). Even though there was only a 125-mg quantity of metronidazole in each tablet, the total dosage of metronidazole in this regimen was 10.5 g, which was higher than the dosages of metronidazole used in most of the double-blind studies involving only metronidazole (Table 2).

Azithromycin has a long half-life and good tissue penetration, so that after a dosage of 500 mg taken once a day for three days, antimicrobial levels of the agent can be found in most tissues for 7 to 10 days (Sefton *et al.*, 1996). This dosage schedule is by far the most convenient regimen that has been evaluated in double-blind studies and should be associated with good patient compliance (see "Compliance").

(3) Dosage guidelines for systemic antimicrobials in periodontal disease

If we note the dosages that were effective in the double-blind studies (Tables 2, 3), as well as the general principles of antimicrobial therapy, certain guidelines can be formulated in regard to dosages to be used for systemic antimicrobial agents in periodontal disease:

(1) Follow the dosage regimens found in the package insert. They are designed to provide a dosage of the agent in all body compartments, and the recommended time between doses has been empirically determined to provide a 24-hour bactericidal/static level of the agent in the tissues.

(2) Because the periodontal pocket is extravascular, the highest possible dose should be used. Take into account the body weight of the patient. Heavier patients need higher dosages. We use 500 mg metronidazole bid for patients weighing between 100 and 200 pounds. We increase the dosage by 250 mg for each 50 pounds over 200 pounds, and decrease it by 250 mg for each 50 pounds under 100 pounds.

(3) Choose a dosage schedule that improves patient compliance. A regimen in which metronidazole is taken twice a day would be better than a regimen in which it is taken three times a day. Doxycycline, and especially azithromycin, have dosage schedules that would promote patient compliance.

(4) Treatment should be as short as possible. There is little need to treat beyond two weeks unless there is evidence of improvement that is short of disease resolution. Most studies with metronidazole have lasted one week. Studies with doxycycline have lasted two weeks or more. Azithromycin is of interest because treatment lasted only three days.

(5) Monitor the efficacy of treatment. If an effect is not apparent by one week, the agent is probably not going to work.

(B) Local delivery devices

(1) Dosages

Local delivery devices make moot a consideration of dosages, since each device brings to the periodontal pocket drug levels that are 10- to 100-fold more than the levels, *i.e.*, from 2 to 10 μg/mL GCF, that can be delivered by the systemic route. For example, with the tetracycline fibers, GCF levels of tetracycline up to 1500 μg/mL can be achieved over a 10-day period (Tonetti *et al.*, 1990). These dosages make tetracycline, which is ineffective *via* the systemic route (Hayes *et al.*, 1992), probably because of its poor activity against anaerobes, now capable of suppressing plaque anaerobes (Goodson *et al.*, 1991; Maiden *et al.*, 1991; Lowenguth *et al.*, 1995). The 25% metronidazole gel that is syringed into the pocket provides up to 450 μg/mL GCF of metronidazole (Stoltze, 1995), and the chip that contains 2.5 mg of chlorhexidine can sustain levels of over 100 ppm in the GCF for at least 7 days (Soskolne *et al.*, 1997).

These are extraordinarily high dosages, and the question becomes, how long can these devices actually maintain these levels in the pockets? Studies with the 25% tetracycline fiber (Goodson, 1994) and a biodegradable chlorhexidine film or chip (Soskolne, 1997) indicated that a seven- to 10-day treatment period is necessary to achieve a clinical result that lasts for several months. Thus, for a device to deliver an effective dosage, it must be retained for at least 7 days. The 25% tetracycline fiber was physically ejected from the pocket within a few days. To maintain the fiber in the pocket, a bandage, such as a cyanoacrylate adhesive (Drisko *et al.*, 1995) or a periodontal dressing (Vandekerckhove *et al.*, 1997), had to be placed over the treated teeth. Even with this precaution, one group reported that 23% of the fibers were extruded from the pockets during a 10-day treatment period (Newman *et al.*, 1994). One could surmise that the bulky fibers simply did not fit into the pocket, and that that was the cause for their ejection. However, when a thin ethylcellulose film containing 20% metronidazole was placed in the pockets, it too was ejected and needed to be anchored to the tooth with a dollop of glass-ionomer cement (Loesche *et al.*, 1996). Likewise, acrylic films containing various antimicrobial agents had to be secured with a periodontal dressing (Addy *et al.*, 1988). In practice, then, non-biodegradable delivery systems need some type of retention mechanism to keep them in place. This mechanism essentially guarantees that the high drug levels will be sustained in the periodontal pocket as long as the device is retained.

This ability of the pocket to eject devices becomes relevant in the context of the biodegradable gel delivery systems that have been commercially developed for doxycycline, metronidazole, and minocycline. The metronidazole gel is almost completely gone after 24 hours (Stoltze, 1995), having decreased from an initial level of 837 μg/mL to 1 μg/mL in a little over a day (Goodson, 1994). Its duration of action was only a few days, so it is not surprising that its manufacturer recommended a second treatment after one week. The half-lives of the minocycline and doxycycline gels would be longer due to the substantivity of the tetracycline family of agents on tooth surfaces (Goodson, 1994; Polson *et al.*, 1997a). But even with this asset, these gels were re-applied a second time (Timmerman *et al.*, 1996; Polson *et al.*, 1997b), and in one study, a retention rate of 85% was obtained when a periodontal dressing was used (Polson *et al.*, 1997a).

The small chlorhexidine chips, *i.e.*, 5 mm x 4 mm x 0.3 mm, containing 2.5 mg of chlorhexidine, reportedly adhere to the tooth and are slowly degraded, thereby maintaining a GCF level of over 100 ppm of chlorhexidine for a seven- to 10-day period. However, no data on the actual number of chips retained for the full seven-day period were reported. In a multicenter study, all sites that remained with 5- to 8-mm pockets were re-treated with the chlorhexidine chips at the three-month visit (Soskolne *et al.*, 1997). Thus, even with this delivery vehicle, re-treatment was deemed necessary.

It seems, then, that the biodegradable gels may need to be re-applied two or more times. This second application negates one of the touted advantages of the biodegradable systems, *i.e.*, that the patient can be treated in a single visit, so that the expense of a second visit to remove the device is avoided. If a gel is lost from the pocket in 1 to 3 days because of ejection and its biodegradability, it would seem all the more prudent to see a patient after a week to ensure that some clinical improvement had occurred.

(2) Choice of agent

The local delivery vehicles bring such high dosages to the pocket environment that Soskolne (1997) has suggested "that the choice of the antibacterial agent is not critical to the clinical result". This is an interesting concept and implies that the nature of the vehicle determines clinical success. The vehicle with the best retention, ease of use, and lowest cost can be used to deliver any agent. While this possibility has not been directly addressed, there is some evidence that the nature of the agent may be important.

Addy *et al.* (1988) randomly assigned patients to a root planing group, a "no treatment" group, and groups that received acrylic strips containing a 50% concentration of either chlorhexidine, metronidazole, or tetracycline. The strips were placed for two consecutive one-week periods in one site with a probing depth > 5 mm *per* patient. A blinded examiner performed the clinical outcome measurements. Inter-group comparisons for both probing depth reductions and gains in attachment showed significantly greater effects for metronidazole and, to a lesser extent, root planing when compared with the chlorhexidine, tetracycline, and "no treatment" groups. These improvements were maintained for at least 14 weeks and were associated, in the metronidazole group, with a sustained reduction in motile organisms and spirochetes. A more detailed bacteriological analysis showed that the tetracycline and metronidazole strips were more effective than chlorhexidine in causing reductions in total anaerobic counts and in the anaerobe/aerobe ratio, but that the tetracycline treatment resulted in the isolation of a large number of resistant organisms (Wade *et al.*, 1992).

Radvar *et al.* (1996) compared scaling and root planing with scaling and root planing plus one of three commercially available products: 2% minocycline gel (Dentomycin®), 25% metronidazole gel (Elyzol®), and the 25% tetracycline fiber (Actisite®), all inserted according

to the manufacturers' instructions. A blinded examiner made the clinical measurements. All antimicrobial treatments produced greater mean improvements in probing depth, attachment level, bleeding on probing, and sites with suppuration, compared with scaling alone, but only the tetracycline treatment was statistically better. Loesche *et al.* (1996) found ethyl cellulose strips containing 20% metronidazole to be more likely to reduce the need for periodontal surgery than ethylcellulose strips containing 20% chlorhexidine.

These data suggest that differences may exist among the agents. However, it may be that the differences observed are really due to characteristics of the vehicle, which determines the bioavailability of agents such as chlorhexidine. Thus, the apparent ineffectiveness of chlorhexidine in the acrylic strips (Addy *et al.*, 1988) should not be compared with its apparent effectiveness in the biodegradable delivery system (Soskolne *et al.*, 1997).

(V) *Where to* Position Antimicrobial Treatments Relative to Debridement

(A) Systemic Agents

For a systemic agent to be effective, it has to be efficiently absorbed in the intestines, circulate in the vascular system to penetrate the extravascular space, and enter the pocket *via* the gingival crevicular fluid. If the GCF flow is about 20 μL/hr (Goodson, 1994), this means that, in a 6-mm pocket, about 480 μL of GCF containing from 1 to 5 μg of the agent arrives *per* day to do battle with the 100,000,000 bacteria that are adhering to the tooth. (These values are based on the assumption that systemic agents achieve GCF levels of 2 to 10 μg/mL, that there is about 1 mg of plaque in a 6-mm pocket, and that 1 mg of plaque contains about 100,000,000 bacteria.) These numbers of bacteria could exhaust the available supply of agent, and could explain why, in the study involving metronidazole in the absence of debridement, a minimal effect of metronidazole was noted (Watts *et al.*, 1986).

It could also explain why periodontal abscesses are sometimes reported in individuals with periodontal disease who are given antibiotics for medical reasons (Topoll *et al.*, 1990). In one report, 10 of 24 patients receiving penicillin developed periodontal abscesses (Helovuo *et al.*, 1993). The prevalence of coagulase-positive staphylococci increased significantly following penicillin therapy, and the prevalence of subgingival Gram-negative enteric rods increased after systemic erythromycin therapy. These authors conclude that, in the absence of mechanical debridement, the exposure of large numbers of subgingival bacteria to low levels of antimicrobials may lead to superinfection with these opportunistic organisms. But it is not just these opportunistic organisms that can overgrow, since many Gram-negative anaerobes isolated from plaque are capable of producing beta-lactamase (Legg and Wilson, 1990; van Winkelhoff *et al.*, 1997). These organisms could be selected for while the patient is on penicillin, and this possibility would be a strong argument against using members of the penicillin family for the treatment of periodontal patients.

To optimize the effect of an antimicrobial agent in the periodontal pocket, one could increase the dosage, as occurs with the local-release delivery vehicles, or decrease the numbers of bacteria on the tooth surface. Both approaches would decrease the ratio of bacteria to antimicrobial agent. If an agent is taken that delivers 5 μg *per* day to the pocket, then the microbe-to-agent ratio is 20,000,000 (100,000,000 divided by 5). If we assume that scaling of the teeth will reduce bacterial levels in a pocket by 90%, *i.e.*, from 100,000,000 to 10,000,000, and the same 5 μg of agent is delivered to the pocket, then the microbe-to-antimicrobial-agent ratio is reduced to 2,000,000 to 1. If the debridement is thorough, such as would occur with root planing, then the bacterial load may be reduced by 99%, giving a microbe-to-agent ratio of 200,000 to 1.

Clearly, after the teeth are debrided, the antimicrobial agent should encounter fewer bacteria in the pocket, and this phenomenon might explain the success of those double-blind studies in which the antimicrobial agent was given after debridement (Tables 2, 3). However, there were clinical improvements in two studies in which metronidazole was given during the first of 4 or 5 visits in which the teeth were debrided (Loesche *et al.*, 1984, 1991). Most of the teeth in these patients would have had high levels of bacteria on their root surfaces when they were exposed to the metronidazole, and the resulting high microbe-to-agent ratio could have minimized the effect of the metronidazole. We evaluated this possibility by repeating the study protocol, but did not dispense the metronidazole until all debridement was completed (Loesche *et al.*, 1992a). An analysis of both studies, in which the only variable was the placement of the metronidazole relative to the debridement, showed that superior results, as measured by reduction in probing depth and gain in attachment, were observed when the debridement preceded the metronidazole (Loesche and Giordano, 1994, 1997). This indicated that the metronidazole was more effective when fewer bacteria were present on the tooth surfaces.

The optimal situation would be for the teeth to be debrided as quickly as possible, and for the patient to take the systemic agent immediately after debridement is completed. This benefit of rapid debridement has been shown in a different context, namely, in the prevention of re-infection with periodontopathogens after treatment with chlorhexidine (Quirynen *et al.*, 1995). Ten patients were randomly assigned either to a group which received

TABLE 4

Levels of Antimicrobial Agents in the Periodontal Pocket and the Effect of Debridement on Microbe/Agent Ratio

Agent	Levels in Pocket	Microbe/Agent Ratio[d] No Debridement	Microbe/Agent Ratio[d] After Debridement (90% reduction)[f]	Retention Time (days)
25% tetracycline	1700 μg/mL[a]	118,000[c]	11,800	7[e]
25% metronidazole	837[a]	238,000	23,800	1
10% Doxycycline	420[b]	476,000	47,600	7[e]
2% Minocycline	165[a]	1,200,000	120,000	4
25% Chlorhexidine	150[b]	1,333,000	133,300	7
Systemic metronidazole	5 μg	20,000,000	2,000,000	—

[a] Goodson, 1994.

[b] Greenstein and Poulson, 1998.

[c] μg/mL divided by 2 to get GCF flow *per* day of 0.5 mL.

[d] Assume that a 6-mm pocket has 100,000,000 bacterial cells in the plaque.

[e] When covered with dressing.

[f] The bacterial levels are decreased from 100,000,000 to 10,000,000.

scaling and root planing in a quadrant at two-week intervals (6 weeks' treatment duration) or to a test group which received all the scaling and root planing in two visits within a 24-hour period. The authors were concerned that periodontopathic bacterial species from the tongue and other soft tissues could re-colonize the teeth. This possibility was reduced by having the subjects in the test group brush their tongues with a 1% chlorhexidine gel, rinse their mouths with a 0.2% chlorhexidine rinse for two weeks, and irrigate their periodontal pockets with a 1% chlorhexidine solution.

When examined one and two months later, the test group showed a significantly greater reduction in probing depths and fewer periodontopathic species compared with the control group (Bollen *et al.*, 1996). Since there were only five subjects in each group, the demonstration of a significant difference indicated that this was a highly effective treatment. In a subsequent study involving 16 patients, the disinfectant procedure was expanded to include, in addition to the tongue brushing and pocket irrigation, a two-month period of rinsing twice a day with a 0.2% chlorhexidine mouthwash and spraying the tonsils with a 0.2% chlorhexidine solution. This multi-modality treatment regimen resulted in greater probing depth reduction, greater attachment gain, and a reduction of spirochetes and *P. gingivalis* in the test group relative to the control group for at least four months after the initial scaling and root planing procedures (Bollen *et al.*, 1998).

This result indicates that all oral reservoirs of the periodontopathic organisms should be treated simultaneously if maximal benefits are to be obtained. Since the species which were significantly reduced were anaerobes, a more efficient way of accomplishing this would be to use a systemic agent, such as metronidazole or doxycycline, which would attack all these reservoirs simultaneously. It also raises the possibility that the local-release delivery devices placed within a pocket may not reach these other oral reservoirs of periodontopathic bacteria. This possibility has been recognized by the concurrent usage of a chlorhexidine mouthrinse in open studies involving the 25% tetracycline fibers (Mombelli *et al.*, 1996; Vandekerckhove *et al.*, 1997).

(B) Local delivery devices

The local delivery devices, when retained in the pocket, can release the agent at levels in excess of 300 μg/mL GCF (Goodson, 1994; Soskolne, 1997; Greenstein and Poulson, 1998), and can achieve microbe-to-agent ratios, *i.e.*, from 118,000 to 476,000 bacteria exposed to each μg of agent, in the absence of debridement, that are comparable with those obtained with systemic agents after a thorough debridement, *i.e.*, 200,000 (see above). This concept is illustrated with data obtained with the 25% tetracycline fiber, which releases about 1700 μg of tetracycline *per* mL GCF (Table 4). About 500 μL of GCF would flow in a 6-mm pocket *per* day, bringing about 850 μg of tetracycline into contact with the 100,000,000 plaque organisms. This translates to a microbe-to-agent ratio of 118,000, which is better than the 200,000-to-1 ratio obtained when systemic agents are combined with a thorough, *i.e.*, 99% efficacy, debridement. The levels of metronidazole released from a 25% metronidazole gel would be about 420 μg *per* day, giving a microbe-to-agent ratio of 238,000; the levels of doxycycline released from the 10% doxycycline gel would have a microbe/drug ratio of 476,000. Both of these values would also be comparable with that obtained with systemic agents and a thorough, *i.e.*, 99% effective, debridement. The 2% minocycline gel and the chlorhexidine film would have microbe-to-agent ratios which are comparable with the ratio observed with systemic agents and a 90% effective debridement (Table 4).

But these favorable ratios exist only when the vehicle is retained in the pocket. The metronidazole gel is gone after 24 to 36 hours (Stoltze, 1995), whereas the tetracycline fiber and the doxycycline gel can be retained for 7 to 10 days with the use of a cyanoacrylate bandage (Drisko *et al.*, 1995; Polson *et al.*, 1997b). Thus, to obtain a complete picture of the agent's efficacy, the length of time that the vehicle remains in place must be accounted for.

In summary, the local delivery devices bring antimicrobial agents into contact with the plaque flora at such high levels that the microbe-to-drug ratio is lower than that which can be obtained by the combination of debridement and systemic agents. These high levels raise a new issue that has profound clinical and cost considerations in periodontal therapy: Do you need to debride the tooth and root surfaces to obtain clinical success, if you can achieve a clinically effective microbe-to-agent ratio in the pocket by placing the device in the pocket?

(C) To debride or not?

The fact that we can even pose this question shows how far our treatment options have expanded in periodontology. Clearly, if a systemic agent is to be used, debridement will greatly improve the clinical outcome to the extent that it should be required. The debridement should be done as quickly as possible prior to the usage of the systemic agent, to provide the most favorable microbe-to-agent ratio. This same consideration should apply to the use of local delivery devices, because it would seem that the agents released by these devices would also benefit from having a more favorable microbe-to-agent ratio as a result of the debridement, *i.e.*, from 11,000 to 133,000 (Table 4). Indeed, the 25% tetracycline fiber (Goodson *et al.*, 1991; Drisko *et al.*, 1995), the 2.5 mg chlorhexidine chip (Soskolne *et al.*, 1997), and the 2% minocycline gels (van Steenberghe *et al.*, 1993; Timmerman *et al.*, 1996; Graca *et al.*, 1997), when combined with scaling and root planing, gave statistically superior results in pocket reduction, when compared with those obtained when a placebo was combined with scaling and root planing.

In other multi-center studies, a 25% metronidazole gel (Ainamo *et al.*, 1992), a 25% tetracycline fiber (Drisko *et al.*, 1995), and a 10% doxycycline gel (Polson *et al.*, 1997b), in the absence of scaling and root planing, were compared with scaling and root planing. The fact that the metronidazole gel and the tetracycline fiber seemed to show equivalency to scaling and root planing, and that the doxycycline gel was statistically superior to scaling and root planing, can best be explained by the favorable microbe-to-agent ratios that can be obtained with these agents (Table 4).

But this explanation cannot account for the significant clinical improvement observed in adult patients who were treated with systemic antimicrobials in the absence of debridement (Table 3) (Lopez and Gamonal, 1998). Forty-six patients with active disease, as measured by at least 2 sites showing ≥2 mm attachment loss, were randomly assigned in a double-blind study to groups which received either metronidazole and amoxicillin for one week, or to a placebo group. At 2 and 4 months after treatment, the antimicrobial group exhibited significantly reduced probing depths and increased attachment levels compared with the placebo group. This difference reflected no change or deterioration in the placebo group compared with an improvement in the treated group. The authors attributed this improvement to the combination of agents used, but for this to be true, groups which received only metronidazole or amoxicillin needed to be included.

This result, as well as those obtained with the local delivery vehicles, challenges the fundamental premise of periodontal treatment—namely, that the teeth have to be physically debrided—and questions the 2000-year-old role of calculus in the etiology of periodontal disease (Mandel, 1995). It will be hard to persuade clinicians that it is not necessary to remove calculus, unless the paradigm has truly changed, *i.e.*, that one may not need to "instrument" the root surface to improve the periodontal condition. An indication that this is possible was observed when the plaque levels of institutionalized teenagers with mental handicaps could be suppressed for a year in the absence of toothbrushing, by applications of a 5% kanamycin paste to the dento-gingival surfaces at five-week intervals (Loesche and Nafe, 1973). Also, in some studies, pocket sites that received only the antimicrobial agent showed improvement comparable with that found in sites receiving scaling and root planing (Lindhe *et al.*, 1983; Addy *et al.*, 1988).

(VI) *How Do We Know that an Antimicrobial Agent Works?*

An antimicrobial agent works if it improves the health of the patient. The types of clinical studies reported in the periodontal literature will be discussed in terms of their design, and as to whether their outcomes reflected tangible benefits to the patient.

(A) Open vs. double-blind studies

The most common clinical studies are open studies, in which either the investigator or the patient or both know what treatment the patient received. Open studies can introduce various biases and confounders which are not randomly distributed between/among the treatment groups, and these could lead to misinterpretation of the clinical results. Consider the situation in an open study involving patients with LJP, or with refractory periodontitis, in which the probing depths and attachment levels

are measured before and after treatment. The attachment level prior to treatment for a given pocket is exactly 5.5 mm, halfway between the 5-mm and 6-mm markings on the periodontal probe. The examiner knows that the patient hasn't yet been treated, and he may "round up" the 5.5 mm to 6 mm for the baseline recording. After treatment, the attachment level remains exactly at 5.5 mm, but since the examiner knows that the patient has been treated, s/he may "round down" the reading to 5 mm. A 1-mm gain in attachment appears on paper, when there has been no change in the actual attachment level.

This type of unintentional bias on the part of the examiner means that caution must be exercised in the interpretation of positive results from open studies. It was open studies that showed a benefit of tetracycline treatment in LJP patients (Slots and Rosling, 1983; Lindhe and Liljenberg, 1984; Mandell *et al.*, 1986; Novak *et al.*, 1991), whereas the only double-blind study in LJP patients showed no advantage for a two-week treatment with doxycycline (Asikainen *et al.*, 1990). If tetracycline was effective, as the open studies indicated, then doxycycline should also have been effective. Subsequently, two groups (Saxén and Asikainen, 1993; Gunsolley *et al.*, 1995) have reported that debridement alone was adequate to manage LJP patients, essentially confirming the results of the double-blind study, which showed no benefit of doxycycline over debridement.

While open studies can provide insights into the design of definitive studies, they should not serve as the basis for treatment decisions. The medical literature is replete with open studies which showed the therapeutic efficacy of medications that were subsequently proven, in double-blind studies, to be ineffective (Chalmers *et al.*, 1983). Double-blind studies in which neither the patients nor the investigators are aware of the treatments being rendered are the "gold standard" in clinical trials, because any biases, measurement errors, and other confounders are randomly distributed between/among the treatment groups. It is for this reason that only double-blind clinical trials are featured in this review (Tables 2, 3).

An important exception would be studies involving the use of vehicles or devices that can be placed within the periodontal pocket in which the clinical trials were single-blind, *i.e.*, the patients were randomly allocated to the treatments, and a blinded examiner measured the treatment outcomes. Given the split-mouth design of these studies, and the nature of the delivery agent, *i.e.*, a fiber, film, or gel delivered to the treatment site, it would be difficult to keep either the patient or the treating clinician ignorant of which sites were treated with the agent. However, the external examiner, if properly shielded from the treatment events, should be able to record outcome measurements objectively, thereby increasing the validity of these studies.

(B) True vs. surrogate clinical outcomes

(1) Tooth loss, a true clinical outcome

The best clinical outcome for a periodontal patient following treatment would be the retention of formerly diseased teeth for a lifetime. The measurement of tooth loss following treatment would be a clinical outcome that would be appreciated by the patient and would meet the definition of a true clinical endpoint (Hujoel and DeRouen, 1995). Several clinicians have described the long-term survival of teeth following periodontal treatment in their private practices (Hirshfeld and Wassermann, 1978; McFall, 1982; Meador *et al.*, 1985). These case reports indicate that about 73 to 83% of patients retain all or most of their teeth as a result of treatment. Accordingly, they provide estimates on tooth loss rates following debridement/surgical treatments which can be used to evaluate new or different treatment modalities. These studies also identified teeth with deep pockets, furcation involvement, extensive bone loss, or marked mobility as having a questionable or "hopeless" prognosis because they did not respond to treatment.

There appears to be only one clinical trial that has used tooth loss as an outcome variable (Hujoel *et al.*, 1997). This is because of the prohibitive expense of conducting studies in which large numbers of subjects would have to be followed for several years for this outcome to be assessed. The utility of tooth loss as an outcome in many studies is undermined by the fact that the "hopeless" teeth, whose response to treatment could be a sensitive indicator of an effective treatment, have often been extracted prior to any treatments. This means that secondary, or surrogate, clinical outcomes are used to evaluate the success of treatment in these studies. The most widely used surrogate outcomes are reduction in probing depth and gain in attachment, although as many as 153 surrogate endpoints have been reported in the periodontal literature (Hujoel and DeRouen, 1995).

(2) Changes in probing depth and attachment levels (surrogate outcomes)

Improvements in probing depths and attachment gain were introduced as clinical endpoints in the 1970s (Kaldahl *et al.*, 1993), and are based on observations that teeth with deep pockets and high levels of attachment loss are likely to have periodontal disease, to be loose, and, if untreated, are at risk to develop abscesses requiring extraction. Any improvements in these parameters would be expected to restore periodontal health. Thus, they appear to be reasonable surrogates for lost teeth, but the relationship between probing measurements and tooth loss is unknown (Hujoel and DeRouen, 1995).

Hujoel *et al.* (1997) sought to validate probing attachment measurements against tooth loss in older patients participating in a clinical trial involving education materials, oral hygiene instructions, periodic prophylaxis, and the use of chlorhexidine rinses. There was a strong statistical correlation between loss of attachment and tooth loss. However, in a logistic regression analysis, the loss of attachment explained only 6% of the tooth loss, which is far below the accepted guideline that a valid surrogate should explain at least 50% to 75% of the true endpoint. This indicated that probing depths and attachment levels do not measure the potential for tooth loss. Rather, they seem to be measuring disease progression that falls short of tooth loss, and whether they are valid surrogate outcomes for tooth loss, or for measuring the efficacy of an antimicrobial agent, remains to be determined.

The use of nonvalidated surrogate outcomes in medicine, especially in chronic diseases, has on occasion led to mistakes, such as the use of drugs to reduce ventricular arrhythmias (Hallström, 1992). Ventricular arrhythmias are associated with increased mortality, so drugs which reduced arrhythmias were sought. The drugs which successfully suppressed the cardiac arrhythmias were estimated to have caused about 8,000 deaths *per* year before they were withdrawn from use. The concern then, to quote Hujoel and DeRouen (1995), is "in the absence of validated surrogates, the choices in trying to obtain definitive answers are to design studies that use true endpoints (such as tooth loss) which may result in large, long and expensive studies; or to continue using nonvalidated surrogates and pray that they won't lead to the kind of incorrect and even dangerous conclusions observed in other areas."

(3) Sample size determinations in clinical trials

The use of probing measurements as an outcome has an effect on the sizes of the treatment groups. The measurement of attachment levels has a standard deviation of ± 1 mm (Pihlstrom, 1992), and this measurement error, *i.e.*, 33% in a 6-mm pocket, decreases the ability to show a treatment effect between/among groups. As a result, it would increase the need for a larger sample size for statistical significance to be shown. Power calculations indicate that about 25 to 30 patients would be needed in each treatment group, to have a difference of 1 mm in attachment level to be significant between/among the groups. In the majority of the clinical studies, there were 15 or fewer subjects in each treatment group, so that only a very obvious difference between/among the groups would be significant. Thus, the possibility of a Type 2 error or false-negative result is great, *i.e.*, reporting no treatment effect when indeed there may be one.

This possibility of a Type 2 error can be illustrated by a double-blind study of systemic metronidazole in individuals with mental retardation. In this study, the authors concluded that "the use of adjunctive metronidazole in a population of retarded adolescents provides no additional benefits to conventional treatment alone" (Clark *et al.*, 1983). Twenty-three subjects were included, and the data on attachment change were reported on two sites *per* patient. If power calculations are applied to the results shown in Table 2, page 661 of that report, then only 15 patients *per* group, or a total of 30 subjects (60 sites), were needed to have an 80% probability of showing a 5% significance between/among the groups. Thus, with a few more subjects, the study would have had enough power to reject the null hypothesis that there were no differences between/among the treatment groups. As it was, the observed trend indicated that the one week of metronidazole treatment was beneficial to these subjects.

The importance of sample size was demonstrated in another study involving metronidazole (Watts *et al.*, 1986). In this investigation, no debridement procedures were provided, and the patients were randomly assigned to either the metronidazole or the placebo group. The study was stopped after two years, because all the dropouts were in the placebo group, so the 13 patients in the metronidazole group were compared with only seven in the control group. The trial demonstrated a "clear but limited difference" in favor of the metronidazole which was found "despite the imbalance in recruitment to experimental and control group, a tendency which could have contributed to possible Type 2 error, that is failing to find a difference which really existed."

The problem of small sample size can be partially addressed by the statistical technique known as meta-analysis. The meta-analysis represents a re-analysis of published data in which results from several studies are combined if they meet predetermined inclusion criteria. The added statistical power obtained with a larger sample size then improves the chances that statistical significance can be shown where previously none was found. Meta-analysis has been applied to published studies of tetracycline, and it was concluded that there was no scientifically valid evidence that tetracycline was of value in periodontal treatment (Hayes *et al.*, 1992). A meta-analysis of metronidazole showed that metronidazole was effective in reducing pocket depths and increasing attachment levels in pockets > 4 mm for several months after treatment had been completed (Elter *et al.*, 1997).

The small sample size often reflects the lack of funding for clinical studies involving generic agents such as tetracycline, metronidazole, minocycline, and doxycycline. No commercial sponsor will invest resources for an agent in which there is no proprietary control. The slow-release delivery systems developed for local treatment

within a pocket are under patent protection and have resulted in a considerable investment by their developers in multi-center clinical trials. These studies are planned in consultation with the FDA, and power calculations are done so that adequate sample sizes are achieved. A new problem in data interpretation can emerge, since now the difference between/among the treatment groups can be statistically significant but may be of minimal clinical relevance, *i.e.*, differences of 0.2 to 0.3 mm gain in attachment between/among treatment groups (Ainamo *et al.*, 1992; Soskolne *et al.*, 1997).

(4) Elimination and/or reduction of the periodontopathogens from the plaque (surrogate outcomes)

If specific periodontopathic bacteria are etiologically involved in periodontal disease, then the efficacy of treatment could be assessed by the disappearance of this organism(s) from plaque samples. The elimination of a pathogen from the host tissue is a valid clinical outcome in medicine, and such an outcome would align periodontal treatments with the established protocols of infectious disease. This assumes that the correct periodontopathogen is being monitored. But which organism is the correct one? Most likely, periodontal disease is a polymicrobial infection, and therefore no single species can be considered as "the periodontopathic species". Our previous discussion indicated that the majority of the evidence implicated the overgrowth of a limited number of anaerobes as the most likely periodontopathogens, so that the monitoring of these bacteria could be used to measure the efficacy of antimicrobial treatments.

Most anaerobes cannot be reliably or inexpensively monitored by culture methods. But the levels of spirochetes can be determined at chairside by either darkfield or phase-contrast microscopy. Many antimicrobial agents can reduce the levels of spirochetes in plaque samples, sometimes to undetectable levels, but with time they return, albeit at lower levels. The most successful treatments in suppressing spirochetes have been described by Keyes and colleagues (Keyes *et al.*, 1978; Rams *et al.*, 1984). After the initial suppression of the spirochetes by debridement and systemic antimicrobials, the patients are instructed to brush their teeth and to irrigate any periodontal pockets with various antimicrobial agents. The levels of spirochetes in the plaque sample are then used to monitor the efficacy of treatment, and to adjust the intensity of the antimicrobial regimen.

Other groups have monitored treatment efficacy by the reduction of black-pigmented species, such as P. *gingivalis* and P. *intermedia*, from plaque samples and observed in most cases a significant reduction in levels that lasted for several months (Loesche *et al.*, 1984, 1991, 1992a; Walker and Gordon, 1990; Kulkarni *et al.*, 1991; van Winkelhoff *et al.*, 1992; Socransky and Haffajee, 1993; Haffajee *et al.*, 1995; Mombelli *et al.*, 1996), Even when all the teeth were debrided within a 24-hour period and chlorhexidine preparations were applied to the pockets, tongue, oral mucosa, and tonsils, P. *gingivalis* was eliminated from only six of 10 patients for up to 2 months (Bollen *et al.*, 1998).

Several investigators have used the decline in plaque levels of A. *actinomycetemcomitans* as a means of measuring the efficacy of treatment. In these open studies, tetracycline was combined with surgery (Mandell *et al.*, 1986; Mandell and Socransky, 1988; Renvert *et al.*, 1996) or was given from three to eight weeks (Slots and Rosling, 1983; Christersson and Zambon, 1993) without A. *actinomycetemcomitans* being eliminated from all plaque samples. Only a combination of metronidazole and ampicillin, again in open studies, has been able to suppress A. *actinomycetemcomitans* in the majority of plaque samples (van Winkelhoff *et al.*, 1992).

The difficulty in eliminating spirochetes or A. *actinomycetemcomitans*, P. *gingivalis*, and P. *intermedia* from plaques suggests that the elimination of these species is not a realistic treatment outcome. Many, if not all, of the periodontopathic bacteria are so prevalent in plaque samples that they could be considered as members of the normal flora. They could also be living in reservoirs such as the tongue and tonsils. A systemic agent might have access to all these locations, but a locally delivered agent probably would not. This would be a disadvantage for the locally delivered agents and is the reason the chlorhexidine rinse is recommended when tetracycline fibers are used (Flemmig *et al.*, 1996; Mombelli *et al.*, 1996; Vandekerckhove *et al.*, 1997).

This persistence of the periodontopathic species probably relates to the underlying microbial ecology of the plaque and the relationship of these species to the host. Rosebury (1962) introduced the concept of an amphibiotic state to describe those infections attributed to the overgrowth of bacterial species that are normally present in the indigenous flora, but at low levels. Certain changes in or on the mucous membranes allow these species to be selected for, and as a result of this overgrowth, they cause an endogenous infection, resulting in clinical disease. Medical examples of endogenous infections would be the several types of diarrhea diseases that occur as a result of malnutrition, vaginal infections due to yeast, superinfections that follow the usage of antibiotics, *e.g.*, a *Clostridium difficile* infection after the use of clindamycin.

Dental caries would be an endogenous infection due to the selection of the mutans streptococci by frequent sucrose ingestion (Loesche, 1993a). It is likely that periodontal disease is an endogenous infection due to the

overgrowth of anaerobic species when plaque accumulates at the dento-gingival margin and in periodontal pockets. An additional selection factor for periodontopathic bacteria would be their ability to utilize nutrients that are made available as a result of tissue inflammation (Loesche, 1993b). Host products such as hemin, menadione, progesterone, estradiol, acetylmuramic acid, spermine, alpha-2 globulin, and ceruloplasmin seem to be essential growth factors for P. *gingivalis*, B. *forsythus*, T. *denticola*, and P. *intermedia*. In a sense, these organisms are host-dependent for their nutrients and, as a result, are very difficult to eliminate from plaques. Because the periodontopathic species can be considered as amphibiotic, it will be difficult to eliminate them from plaque samples. A more realistic treatment endpoint would be to reduce the levels of the periodontopathic species to below a certain threshold level, but these levels are not reliably known.

(5) Reduced need for surgery

In our first metronidazole double-blind study, we observed a significant reduction in probing depth and gain in attachment following debridement in the metronidazole group relative to the placebo group (Loesche *et al.*, 1984). When these patients were re-examined after one year to determine how long the beneficial effects of the metronidazole would last, many of the pockets which had shown clinical improvement had subsequently been eliminated by surgery. This meant that the benefits measured by probing depths and attachment level measurements were either so fleeting that they could not prevent surgery from being performed, or that surgery was performed because of the residual pocket depths, even though the tissue appeared healthy.

This situation led us to re-assess what would be a meaningful clinical outcome both for the patient and for the clinical investigation. Since surgery can be a deterrent for the patient, due to both cost and perceived discomfort (Meador *et al.*, 1985; Matthews and McCulloch, 1993), a decision was made to determine whether successful treatment would reduce the need for surgery. We also stopped the *a priori* extraction of "hopeless teeth" so that these teeth could be used as indicators to monitor the effect of treatment. These outcome measurements had the immediate advantage of reducing the cost and discomfort of surgery to the patient, and because teeth were at least initially spared from extraction, approached the definition of a true outcome for the patient. This reduced need for surgery could simply be surgery delayed, and as a precaution against the premature report of success based on an ephemeral outcome, we withheld the publication of our findings until we had one or more years of follow-up on these patients (Loesche *et al.*, 1991, 1992a, 1996).

The decision as to whether a tooth needs surgery or extraction is made daily in clinical periodontology, yet the parameters for that decision-making process have never been standardized. We established guidelines for the determination of surgical need, or extractions, which were based upon probing depths and attachment levels; the presence or absence of bleeding or exudate; the nature of the root topography and bony defects as seen in radiographs; the nature and extent of any furcation involvement; the magnitude of tooth mobility; and whether access would be adequate for thorough root instrumentation (Loesche *et al.*, 1991, 1992a). Using these guidelines, two clinicians, each with over 30 years of clinical experience, independently examined the patients, and their findings were used to determine the measurement error (Loesche *et al.*, 1996). The inter-examiner correlation coefficient for surgical needs was r = 0.94, and the intra-examiner correlation coefficient was r = 0.98. The tooth-by-tooth % agreement was 90%, and the kappa statistic was 0.85.

The reason for this high level of agreement could be the convergence of so many clinical parameters on a single yes/no decision, *i.e.*, probing and attachment measurements, bleeding on probing, mobility, furcations, tooth type, tissue appearance, and bone levels on radiographs. As such, this measurement is much richer than the one-dimensional attachment levels or probing depth scores that are usually reported as outcome variables in clinical trials of antimicrobial agents.

(C) The interpretation of studies which use surrogate endpoints

(1) Clinical relevance of surrogate endpoints

How do you interpret the success of clinical trials that rely upon surrogate outcomes such as gain in probing attachment levels, if no one knows what this gain means in terms of tangible benefit to the patient? Presumably, a gain in attachment level results in increased retention of the tooth in the mouth, but Hujoel *et al.* (1997) concluded that "probing attachment levels can be ruled out as being anything more than a weak surrogate marker for tooth mortality". While there is no doubt that improvements in attachment levels as a result of treatment are beneficial, the meaning of this benefit cannot be assessed by the probing measurements themselves.

For example, Quee *et al.* (1987) showed that the Rodogyl®-treated group compared with the placebo group (both groups received scaling and root planing) exhibited a significant whole-mouth average gain in attachment of 0.67 mm. Yet they cautioned that this difference was not necessarily of biological significance and speculated whether the placebo group would have undergone periodontal breakdown sooner than the

TABLE 5

Effect of Scaling/Root Planing and Either Metronidazole or Placebo on Clinical Outcomes

	Scaling & Root Planing & Metronidazole (n = 33 patients)	Scaling & Root Planing & Placebo (n = 39 patients)	Significance
	Number of Teeth Needing Surgery/Patient		
At baseline	15.1 ± 6.4[c]	13.4 ± 8.0	
At completion	8.0 ± 6.3	11.0 ± 7.6	
Change	7.1 ± 6.1	2.4 ± 5.7	P = 0.002[a]
Sites with Initial Probing Depth	Change in Probing Depths/Patient		
≤ 3 mm (21-121)[d]	- 0.04 mm	+ 0.09 mm	0.28[b]
4 to 6 mm (16-74)	- 0.96	- 0.78	0.16
≥ 7 mm (1-51)	- 2.32	- 1.63	0.003
Sites with Initial Probing Depth	Change in Attachment Levels/Patient		
≤ 3 mm (21-121)	- 0.01 mm	- 0.23 mm	0.03[b]
4 to 6 mm (16-74)	0.58	0.29	0.01
≥ 7 mm (1-51)	1.22	0.75	0.02

[a] Two-factor ANOVA.

[b] Weighted ANOVA.

[c] Average ± standard deviation.

[d] Range of sites in each category *per* patient.

Adapted from Loesche and Giordano, 1994.

Rodogyl®-treated group. That apparently would have been a clinically relevant outcome, since it indicated that the gain in attachment levels halted or slowed disease progression. McCulloch *et al.* (1990) used disease progression as their clinical outcome, *i.e.*, doxycycline reduced the rate at which refractory patients developed abscesses and lost teeth.

The double-blind clinical trials of locally delivered tetracycline (Drisko *et al.*, 1995), minocycline (van Steenberghe *et al.*, 1993), doxycycline (Polson *et al.*, 1997b), and chlorhexidine (Soskolne *et al.*, 1997) were sufficiently powered with large numbers of subjects, so that average whole-mouth differences of 0.2 to 0.6 mm in attachment levels between the test and placebo groups were statistically significant. But how do you assign clinical significance to these modest gains in attachment? Soskolne (1997) speculated that by decreasing loss of attachment, "controlled subgingival antibacterial therapy should reduce the sites needing surgery by 50%". This implies that a reduction in surgical needs would be a tangible attribute of the clinical relevance of improvements in attachment levels.

(2) Reduced need for surgery as a relevant clinical outcome

A reduction in surgical needs has been used as a clinical endpoint in three double-blind studies involving metronidazole (Loesche *et al.*, 1991, 1992a, 1996). A sparing of teeth from extraction is a true endpoint, since it prevents the immediate loss of the tooth and whatever adverse consequences this might have for masticatory function and esthetics. A reduction in the need for periodontal surgery has an immediate clinical relevance, since it provides the patient with a tangible benefit, the avoidance of surgery, which benefit could encourage the patient to continue treatment and/or allow him/her to afford treatment. If periodontal disease is a risk factor for heart disease (DeStefano *et al.*, 1993; Mattilla *et al.*, 1995; Beck *et al.*, 1996; Loesche *et al.*, 1998a), then anything that encourages the patient to obtain periodontal treatment would be an important benefit.

The combined data from the first two studies showed that, in terms of surgical need, there was an average reduction of 7.1 teeth *per* patient in the metronidazole group, and of 2.4 teeth *per* patient in the positive control group (difference significant at the p = 0.002 level; Table 5) (Loesche and Giordano, 1994). This reduction was associated with a significant reduction in pockets that were initially > 6 mm, and a significant gain in attachment at all pocket depths compared with the control (Table 5). In the third study, the goal of treatment was to determine how much surgical need could be avoided by a protocol that used either metronidazole or doxycycline in the first round of treatment, with the type of treatment rendered thereafter—*e.g.*, re-treatment with systemic agents or local delivery of metronidazole or chlorhexidine—dependent upon the number of teeth still requiring surgery or extraction. Ninety patients, including patients with refractory and early-onset periodontitis, who at entry into the study had an average of 8.7 teeth/patient in need of access surgery or extractions, completed the treatment phase of the study. Overall, 81% of these patients and 93% of the 640 teeth initially recommended for access surgery did not need this surgery. Sixty-six percent of the "hopeless" teeth, initially recommended for

extraction, needed neither extraction nor access surgery (Table 6).

TABLE 6

Ability of Debridement Plus Systemic and/or Locally Delivered Antimicrobial Treatments to Reduce Surgical Needs

	No. of Teeth/ Patient	Total Number of Teeth Needing Surgery	Extractions	Total	No. of Patients to Maintenance
At baseline, n = 90 patients	23.7	640	143	783	0
At completion, n = 90	23.3	45	48	93	73 (81%)[b]
Teeth spared surgery		595 (93%)[a]	95 (66%)[a]	690 (88%)[a]	

[a] Percentage of teeth spared either the surgery or extractions that were recommended at baseline.

[b] Percentage of patients who entered maintenance phase without needing surgical treatment.

Adapted from Loesche *et al.*, 1996.

These studies indicate that most surgical procedures could be prevented by a combined debridement/antimicrobial approach. This begs the question, "How long should a treatment benefit last in order for it to be considered a real benefit"? In the first two studies, the patients were followed for at least 18 months to determine if the observed reduction in surgical needs could be sustained (Loesche *et al.*, 1991, 1992a). The patients who had been treated with metronidazole fared better than those who had been in the positive control group. Sixteen percent of the patients in the positive control group relapsed, in that they had 4 or more teeth needing surgery, compared with 8% of the patients in the metronidazole group (Loesche and Giordano, 1997). At the entrance to the maintenance phase, there were 213 teeth that had been spared from surgery or extraction in the metronidazole patients. During the next 18 months, 19 of these teeth needed surgery or extraction, while the other 196 teeth remained healthy. In the positive control patients, 97 teeth relapsed. Thus, even in the maintenance period, a residual benefit of the initial metronidazole treatment was observed, indicating that a sustained benefit had been conferred by the metronidazole treatment.

In the third metronidazole study, 73 of the 90 patients who completed the study did not receive any surgery or extraction during the active treatment phase, so that one might expect that this legacy of surgical need would come due sometime during the maintenance phase. Eighty-two of these 90 patients have been seen at least once in the maintenance phase; 63 patients have been examined twice, and 50 have been examined three times. Very few teeth have needed either surgery or extraction during the first 4 years of recall (Table 7). These 82 patients were initially treatment-planned as having 8.9 teeth/patient needing surgery or extraction. In reality, only 9% of these teeth actually received surgery or extraction during the active treatment phase, and another 13% received surgery or extraction during the following 1 to 4 years of the maintenance phase. Forty-five patients (56%) have not needed surgery, and eight patients (10%) have accounted for about 50% of the teeth needing surgery or extraction. These results indicate that over 70% of teeth that were initially spared surgery/extraction have remained free of surgical intervention during the first 1 to 4 years of the maintenance phase. This would appear to be a tangible benefit to the patients, and indeed the patients indicate this in their responses on a questionnaire evaluating patient satisfaction with treatment.

TABLE 7

The Number of Teeth Needing Surgery or Extraction in the 1- to 5-year Period Following Initial Antimicrobial Treatment (Loesche *et al.*, 1996)

Treatment Stages	No. of Patients	No. of Teeth/ Patient	Number of Teeth/Patient Needing Surgery	Extractions	Total
At baseline	82	23.7	7.1	1.7	8.9
At completion of treatment	82	23.3	0.3	0.8	1.1
At 1st recall	82	22.8	0.3[a]	0.8[a]	1.1[a]
At 2nd recall	63	22.5	0.3	1.1	1.4
At 3rd recall	50	22.4	0.4	1.2	1.6

[a] Cumulative number of new teeth needing surgery or extraction during the recall period (the 1.1 total teeth/patient needing surgery or extraction at completion of treatment is not included in this number).

(D) Patient compliance

If antimicrobial agents are to be used in the treatment of periodontal infections, their efficacy will be dependent upon patient compliance. This is an important issue when the results of treatment are evaluated, because an unsatisfactory result could indicate either an ineffective agent or a non-compliant patient. An effective agent that is not used by the patient is of little value.

(1) Systemic agents

Patient compliance with the unsupervised usage of prescription medication is a serious concern in medicine (Greenberg, 1984; Eisen *et al.*, 1990) and is a major confounder of clinical trials (Freedman, 1990). Patient non-compliance is a function of socio-economic factors (Nagasawa *et al.*, 1990), the clarity of the instructions (Stewart and Caranasos, 1989), and the daily frequency at which the medication should be taken (Cockburn *et al.*, 1987; Eisen *et al.*, 1990). As an example of the latter, non-compliance with antihypertensive medications was 41% on a 3x daily regimen, but only 16% on a once-a-day regimen (Eisen *et al.*, 1990). This would indicate that in studies evaluating systemic antimicrobial agents in periodontal disease, patient compliance will decrease as a function of the number of tablets that should be taken daily and the length of the treatment. The best results should be obtained with antimicrobials that are the easiest to take.

This consideration would support the usage of doxycycline, which needs to be taken only once a day for at least two weeks (McCulloch *et al.*, 1990; Loesche *et al.*, 1996), and would encourage more studies with azithromycin, which appears to be effective after one tablet is taken daily for only three days (Sefton *et al.*, 1996). It would discourage the usage of medications that need to be taken multiple times a day, such as tetracycline (four times a day), Rodogyl® (two tablets three times a day), and combinations of metronidazole and amoxicillin (two tablets three times a day).

Bottles with metronidazole carry a label warning the patient not to consume alcoholic beverages, which label is likely to discourage compliance. We developed a spirochete and BANA assay to measure patient compliance with metronidazole (Loesche *et al.*, 1993). We gave patients metronidazole under supervision and demonstrated that the plaque spirochetes either could not be detected or had greatly decreased at 1 and 8 days after patients stopped taking the medication. We chose spirochetes as our indicator organism, since they are sensitive *in vitro* to < 0.1 μg/mL metronidazole (Cheng *et al.*, 1985), and these levels are easily achievable in the GCF (Britt and Pohlod, 1986). Spirochetes are not known to be resistant to metronidazole, so that if their levels remained unchanged in plaque samples in patients assigned to the metronidazole group, the patient was considered non-compliant. We also monitored compliance by the conversion of plaque samples from BANA-positive to BANA-negative, on the basis that the BANA-positive species, P. *gingivalis*, B. *forsythus*, and T. *denticola*, are sensitive to low levels of metronidazole and are not known to have developed resistance to this agent (Listgarten *et al.*, 1993; Larsen and Fiehn, 1997).

We then evaluated patient compliance by retrospectively monitoring the response of plaque spirochetes and the BANA test in patients involved in a double-blind clinical trial in which the metronidazole was taken 250 mg tid for one week (Loesche *et al.*, 1993). Seven of the 18 patients in the metronidazole group appeared to be non-compliant, since the levels of spirochetes showed no change or actually increased, and the BANA test remained positive (Loesche *et al.*, 1993). All 18 patients claimed to have taken their medication and brought back empty medication bottles. When the clinical data were re-analyzed according to whether the patient was compliant or non-compliant, the number of teeth requiring periodontal surgery was decreased by 8.3 teeth in the compliers, but by only 3.6 teeth in the non-compliers.

These findings suggested that 40% of the metronidazole patients were non-compliant, but despite this, and the small sample size (n = 18 patients), the reduction in the need for surgery for the metronidazole patients compared with the placebo patients was significant (Loesche *et al.*, 1991). This indicated that metronidazole was an extremely effective drug in treating anaerobic periodontal infections. For compliance to be improved, the metronidazole can be taken 500 mg twice a day, or a slow-release metronidazole tablet (G.D. Searle, Chicago, IL, USA), to be taken only once a day, can be used.

(2) Locally delivered agents

The development of local delivery vehicles solves the problem of patient compliance, since the vehicles are professionally placed, thereby negating any reliance upon the patient to take the medication. However, the duration of treatment effect, which could be considered as a manifestation of compliance, will vary according to whether these vehicles are retained for the duration of treatment. If these vehicles are lost, then the pocket was not exposed to the full dosage, and in a sense the patient could be considered as non-compliant.

This argument cannot be applied to the biodegradable vehicles, because if the vehicle is not present at one week, it could have been lost from the pocket as a result of those forces which eject fibers and films, or because it

was lost *via* its intended biodegradation. It is not known if vehicles that are in the process of being biodegraded are lost more quickly than vehicles that are not biodegradable. In this regard, the 10% doxycycline gel was retained in place with a periodontal dressing (Polson *et al.*, 1997a), so that these teeth could be considered, in a sense, compliant. The chlorhexidine-containing chip reportedly is retained for 7 to 10 days (Soskolne, 1997), and pockets so treated could be considered compliant. The 25% metronidazole gel (Stoltze, 1995) and the 2% minocycline gel may not remain *in situ* long enough for the treated pockets to be considered compliant.

(VII) *Changing the Paradigm*

(A) The Antimicrobial Management of Periodontal Disease

According to the Specific Plaque Hypothesis, a measurable amount of periodontal disease is due to the overgrowth of specific bacterial types. This indicates that in the presence of clinical disease and the documented overgrowth of these periodontopathic species, antimicrobial treatments involving both mechanical procedures and chemical agents can be used for short periods of time to achieve long-term effects. The established treatments are based upon a non-specific plaque overgrowth paradigm, which, if therapy is maintained on a periodic basis for a lifetime, can provide satisfactory results for about 80% of periodontal patients. In order for the Specific Plaque Hypothesis to change the existing periodontal treatment paradigm, it would have to offer advantages beyond those provided by the debridement/surgical paradigm.

Both paradigms are antimicrobial, in that debridement is used to limit the numbers of bacteria that accumulate on the tooth surfaces. They differ in the treatment of patients with advanced disease. The Non-specific Plaque paradigm states that, for adequate debridement of the tooth/root surfaces when there are deep pockets, the pockets need to be surgically eliminated, so that the patient can practice good oral hygiene on these newly accessible tooth/root surfaces. The surgery is often accompanied by the use of systemic antimicrobials, to prevent post-operative infections. If the patient cannot maintain clean tooth/root surfaces after the surgical approach, and the diseased pockets return, then systemic antimicrobials are used in a rescue or salvage mode. Thus, in the Non-specific Plaque Paradigm, there would appear to be no contra-indications for the use of antimicrobial agents, provided that this use coincides with the surgical procedures, or is used when surgery fails (Fig.) (Genco, 1991; Goodson, 1994; Anonymous, 1996a,b).

The Specific Plaque paradigm, in contrast, recommends that the short-term usage of antimicrobial agents, in combination with debridement, should precede any surgical intervention (Fig.). It requires that one identify the periodontopathic flora prior to any treatment and then use the appropriate antimicrobial agent. Over 20 bacteriological studies have implicated the overgrowth of anaerobic species as being statistically associated with advanced forms of periodontal disease. This finding would call for the short-term usage of antimicrobial agents directed against anaerobic members of the flora. Metronidazole and doxycycline have been shown to be effective in double-blind studies, and the Specific Plaque Hypothesis would recommend their use in patients diagnosed with anaerobic infections. The Specific Plaque Hypothesis

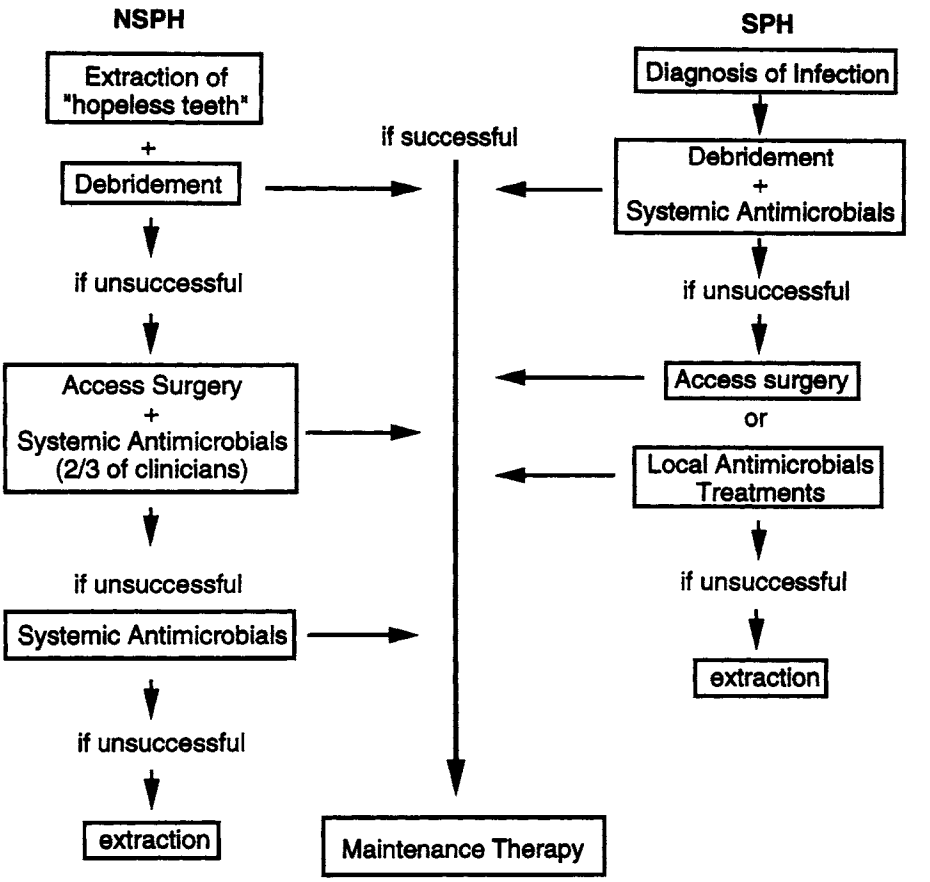

Figure. The non-specific plaque hypothesis (NSPH) *vs.* the specific plaque hypothesis (SPH): same modules of treatment—different sequence.

would reserve the surgical approach for those instances when the antimicrobial approach fails (Fig.).

The two paradigms use the same modules of treatment but differ operationally in the sequencing of these modules. It is this sequencing that has profound implications in the management of periodontal disease. If periodontal disease behaves as a treatable infection, then an antimicrobial approach would reduce the need for labor-intensive surgical procedures, thereby offering the patient a less expensive treatment protocol. This advantage alone should be sufficient to explore treatments based upon the Specific Plaque paradigm. But will a profession embrace a specific infection treatment paradigm after years of apparent success with a debridement/surgical paradigm? The answer is probably "yes", for the following reasons:

The data on the microbial etiology of most forms of periodontal disease are overwhelming and have led to double-blind studies which have shown that advanced forms of periodontal disease can be successfully treated by short-term usage of metronidazole and doxycycline. But information on the success of these agents has not been widely communicated within the dental community, partly because it challenges the traditional debridement/surgical treatment paradigm, and partly because these agents are generic drugs without any industry support. A recent review in the medical literature states that "if metronidazole were a patented antibiotic, the manufacturer would almost certainly be willing to put substantial marketing resources into convincing the clinical community that antibiotic treatment of periodontal disease was less invasive, cost-effective, cosmetically superior, and less risky than surgery" (Hay and Yu, 1999).

While industry support is missing for these generic antimicrobials (although it might be there for azithromycin; Zithromax®), it is there for the local-release delivery vehicles for tetracycline (Actisite®), chlorhexidine (PerioChip®), doxycycline (Atridox®), and metronidazole (Elyzol®). Once the benefits of this local treatment are observed, and the clinicians become comfortable with this usage of antimicrobial agents, then systemic agents will be tried. Eventually, the proper mix of debridement and systemic and locally delivered agents will be developed, and these protocols will become part of the standard of care. This will happen because it will be in the patient's interest for periodontal disease to be prevented and treated, so that the onset of heart disease and other systemic medical conditions can be possibly prevented and/or delayed.

(B) The medical connection

In the late 20th century, the paradigm on the etiology of heart disease is changing to suggest a chronic infection hypothesis (Valtonen, 1991), which would include dental infections (Syrjänen, 1990; Mattilla *et al.*, 1998). At least 10 studies, after the investigators adjusted for many known risk factors for heart disease, have shown a statistically significant association between various forms of cardiovascular disease and dental disease, especially periodontal disease (Beck *et al.*, 1996; Loesche and Lopatin, 1998). Mattilla and colleagues (1995), in a prospective study, found dental disease to be a significant risk factor for the development of a second heart attack which often culminated in death. In a prospective study of a large number of American males, periodontal disease and edentulousness were statistically associated with coronary heart disease, and with death from any cause, after many known risk factors were adjusted for (DeStefano *et al.*, 1993).

Elderly dependent-living US veterans who reported seeing their dentist at least once a year were almost five times less likely to have suffered a cerebral vascular accident, when compared with elderly veterans who did not see their dentist (Loesche *et al.*, 1998b). Elderly veterans who were dentate and whose plaques were BANA-positive, suggesting the presence of the anaerobic species associated with periodontal disease, were twice as likely to have a diagnosis of coronary heart disease than were elderly dentate veterans whose plaques were BANA-negative (Loesche *et al.*, 1998a).

These results suggest that periodontal disease can be considered a risk factor for cardiovascular diseases. If this is so, then it is a modifiable risk factor, and one that will become a primary target for interceptive and preventive strategies. The management of periodontal disease by means of a treatment strategy based on the Specific Plaque Hypothesis is easier and less expensive to implement than one based on debridement/surgical intervention, as espoused by the Non-specific Plaque Hypothesis.

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References

Addy M, Hassan H, Moran J, Wade W, Newcombe R (1988). Use of antimicrobial containing acrylic strips in the treatment of chronic periodontal disease. A three month follow-up study. J *Periodontol* 59:557-564.

Ainamo J, Lie T, Ellingsen BH, Hansen BF, Johansson LA, Karring T, *et al.* (1992). Clinical responses to subgingival application of a metronidazole 25% gel compared to the effect of subgingival scaling in adult periodontitis. J *Clin Periodontol* 19:723-729.

Aitken S, Birek P, Kulkarni GV, Lee WL, McCulloch CA

(1992). Serial doxycycline and metronidazole in prevention of recurrent periodontitis in high-risk patients. J *Periodontol* 63:87-92.

Al-Joburi W, Chin-Quee T, Lautar C, Iugovaz I, Bourgouin J, Delorme F, *et al.* (1989). Effects of adjunctive treatment of periodontitis with tetracycline and spiramycin. J *Periodontol* 60:533-539.

Ames BN (1986). Food constituents as a source of mutagens, carcinogens and anticarcinogens. *Prog Clin Biol Res* 206:3-32.

Ames BN, Magaw R, Gold LS (1987). Ranking possible carcinogenic hazards. *Science* 236:271-280.

Andriole VT (1998). Quinolones. In: Infectious diseases. 2nd ed. Gorbach S, Bartlett J, Blacklow N, editors. Philadelphia: W.B. Saunders Co., pp. 275-289.

Anonymous (1989). In: Newsletter. Vol. 24. Chicago, IL: American Academy of Periodontology, p. 4.

Anonymous (1996a). Systemic antibiotics in periodontics. J *Periodontol* 67:831-838.

Anonymous (1996b). Consensus report. Non-surgical pocket therapy: mechanical, pharmacotherapeutics, and dental occlusion. *Ann Periodontol* 1:581-588.

Ashimoto A, Chen C, Bakker I, Slots J (1996). Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontal lesions. *Oral Microbiol Immunol* 11:266-273.

Asikainen S, Jousimies-Somer H, Kanervo A, Saxén L (1990). The immediate efficacy of adjunctive doxycycline in treatment of localized juvenile periodontitis. *Arch Oral Biol* 35(Suppl):231S-234S.

Barnett ML (1985). Inhibition of oral contraceptive effectiveness by concurrent antibiotic administration. A review. J *Periodontol* 56:18-20.

Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S (1996). Periodontal disease and cardiovascular disease. J *Periodontol* 67:1123-1137.

Bollen CM, Vandekerckhove BN, Papaioannou W, Van Eldere J, Quirynen M (1996). Full- versus partial-mouth disinfection in the treatment of periodontal infections. A pilot study: long-term microbiological observations. J *Clin Periodontol* 23:960-970.

Bollen CM, Mongardini C, Papaioannou W, van Steenberghe D, Quirynen M (1998). The effect of a one-stage full-mouth disinfection on different intraoral niches. Clinical and microbiological observations. J *Clin Periodontol* 25:56-66.

Britt MR, Pohlod DJ (1986). Serum and crevicular fluid concentrations after a single oral dose of metronidazole. J *Periodontol* 57:104-107.

Burtin P, Taddio A, Ariburnu O, Einarson TR, Koren G (1995). Safety of metronidazole in pregnancy: a meta-analysis. *Am J Obstet Gynecol* 172:525-529.

Calia FM, Oldach DW (1998). Macrolide antibiotics. In: Infectious diseases. 2nd ed. Gorbach S, Bartlett J, Blacklow N, editors. Philadelphia: W.B. Saunders Co., pp. 242-259.

Chalmers TC, Celano P, Sacks HS, Smith H Jr (1983). Bias in treatment assignment in controlled clinical trials. *New Engl J Med* 309:1358-1361.

Cheng S-L, Siboo R, Quee TC, Johnson JL, Mayberry WR, Chan ECS (1985). Comparative study of six random oral spirochete isolates. J *Periodont Res* 20:602-612.

Chin-Quee T, Chan EC, Clark C, Lauter-Lemay C, Bergeron MJ, Bourgoin J, *et al.* (1987). The role of adjunctive Rodogyl therapy in the treatment of advanced periodontal disease. A longitudinal clinical and microbiologic study. J *Periodontol* 58:594-601.

Christersson LA, Zambon JJ (1993). Suppression of subgingival *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis by systemic tetracycline. J *Clin Periodontol* 20:395-401.

Christersson LA, Fransson CL, Dunford RG, Zambon JJ (1992). Subgingival distribution of periodontal pathogenic microorganisms in adult periodontitis. J *Periodontol* 63:418-425.

Clark DC, Shenker S, Stulginski P, Schwartz S (1983). Effectiveness of routine periodontal treatment with and without adjunctive metronidazole therapy in a sample of mentally retarded adolescents. J *Periodontol* 54:658-665.

Cockburn J, Gibberd RW, Reid AL, Sanson-Fisher RW (1987). Determinants of non-compliance with short-term antibiotic regimens. *Br Med J* 295:814-818.

DeStefano F, Anda RF, Kahn HS, Williamson DF, Russell CM (1993). Dental disease and risk of coronary heart disease and mortality. *Br Med J* 306:688-691.

Drisko CL, Cobb CM, Killoy WJ, Michalowicz BS, Pihlstrom BL, Lowenguth RA, *et al.* (1995). Evaluation of periodontal treatments using controlled-release tetracycline fibers: clinical response. J *Periodontol* 66:692-699.

Duckworth R, Waterhouse JP, Britton DE, Nuki K, Sheiham A, Winter R, *et al.* (1966). Acute ulcerative gingivitis. A double-blind controlled clinical trial of metronidazole. *Br Dent J* 120:599-602.

Dzink JL, Socransky SS, Haffajee AD (1988). The predominant cultivable microbiota of active and inactive lesions of destructive periodontal diseases. J *Clin Periodontol* 15:316-323.

Eisen SA, Miller DK, Woodward RS, Spitznagel E, Przybeck TR (1990). The effect of prescribed daily dose frequency on patient medication compliance. *Arch Intern Med* 150:1881-1884.

Elter JR, Lawrence HP, Offenbacher S, Beck JD (1997). Meta-analysis of the effect of systemic metronidazole as an adjunct to scaling and root planing for adult periodontitis. J *Periodont Res* 32:487-496.

Fiehn NE, Westergaard J (1990). Doxycycline-resistant bacteria in periodontally diseased individuals after systemic doxycycline therapy and in healthy individuals. *Oral Microbiol Immunol* 5:219-222.

Fitzgerald RJ (1968). Dental caries in gnotobiotic animals. *Caries Res* 2:139-146.

Flemmig TF, Weinacht S, Rudiger S, Rumetsch M, Jung A, Klaiber B (1996). Adjunctive controlled topical application of tetracycline HCl in the treatment of localized persistent or recurrent periodontitis. Effects on clinical parameters and elastase-alpha1-proteinase inhibitor in gingival crevicular fluid. J *Clin Periodontol* 23:914-921.

Freedman LS (1990). The effect of partial non-compliance on the power of a clinical trial. *Controlled Clin Trials* 11:157-168.

Garcia-Rodriguez JA, Garcia-Sanchez JE, Trujillano-Martin T, Garcia-Sanchez E, Garcia-Garcia MI, Fresnadillo-Martinez MJ (1995). In vitro activity of BAY y 3118 and nine other antimicrobial agents against anaerobic bacteria. J *Chemother* 7:189-196.

Genco RJ (1991). Using antimicrobial agents to manage periodontal diseases. J *Am Dent Assoc* 122:30-38.

Goene RJ, Winkel EG, Abbas F, Rodenburg JP, van Winkelhoff AJ, de Graaff J (1990). Microbiology in diagnosis and treatment of severe periodontitis. A report of four cases. J *Periodontol* 61:61-64.

Goodson JM (1994). Antimicrobial strategies for treatment of periodontal diseases. *Periodontol* 2000 5:142-168.

Goodson JM, Tanner A, McArdle S, Dix K, Watanabe SM (1991). Multicenter evaluation of tetracycline fiber therapy. III. Microbiological response. J *Periodont Res* 26:440-451.

Gordon JM, Walker CB (1993). Current status of systemic antibiotic usage in destructive periodontal disease. J *Periodontol* 64:760-771.

Gordon J, Walker C, Hovliaras C, Socransky SS (1990). Efficacy of clindamycin hydrochloride in refractory periodontitis: 24-month results. J *Periodontol* 61:686-691.

Graca MA, Watts TL, Wilson RF, Palmer RM (1997). A randomized controlled trial of a 2% minocycline gel as an adjunct to non-surgical periodontal treatment, using a design with multiple matching criteria. J *Clin Periodontol* 24:249-253.

Greenberg RN (1984). Overview of patient compliance with medication. *Clin Ther* 6:592-599.

Greenstein G, Poulson A (1998). The role of local drug delivery in the management of periodontal disease: a comprehensive review. J *Periodontol* 69:507-520.

Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, *et al.* (1994). Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. J *Periodontol* 65:260-267.

Grossi SG, Genco RJ, Machtei EE, Ho AW, Koch G, Dunford R, *et al.* (1995). Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. J *Periodontol* 66:23-29.

Gunsolley JC, Califano JV, Koertge TE, Burmeister JA, Cooper LC, Schenkein HA (1995). Longitudinal assessment of early onset periodontitis. J *Periodontol* 66:321-328.

Gupta S, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camon AJ (1997). Elevated *Chlamydia pneumoniae* antibodies, cardiovascular events and azithromycin in male survivors of myocardial infarction. *Circulation* 96:404-407.

Gusberti FA, Syed SA, Lang NP (1988). Combined antibiotic (metronidazole) and mechanical treatment effects on the subgingival bacterial flora of sites with recurrent periodontal disease. J *Clin Periodontol* 15:353-359.

Haffajee AD, Socransky SS (1994). Microbial etiological agents of destructive periodontal diseases. *Periodontol* 2000 5:78-111.

Haffajee AD, Dibart S, Kent RL Jr, Socransky SS (1995). Factors associated with different responses to periodontal therapy. J *Clin Periodontol* 22:628-636.

Haffajee AD, Cugini MA, Tanner A, Pollack RP, Smith C, Kent RL Jr, *et al.* (1998). Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. J *Clin Periodontol* 25:346-353.

Hallström A (1992). Antiarrhythmic drugs: before and after the CAST. WA *Public Health* 10:48-50.

Hay JW, Yu W (1999). Drug patents and prices: can we achieve better outcomes? In: Measuring the prices of medical treatments. Triplett J, Helms R, editors. Washington, DC: Brookings Institution Press (in press).

Hayes C, Antczak-Bouckoms A, Burdick E (1992). Quality assessment and meta-analysis of systemic tetracycline use in chronic adult periodontitis. J *Clin Periodontol* 19:164-168.

Helovuo H, Hakkarainen K, Paunio K (1993). Changes in the prevalence of subgingival enteric rods, staphylococci and yeasts after treatment with penicillin and erythromycin. *Oral Microbiol Immunol* 8:75-79.

Hirshfeld L, Wassermann B (1978). A long-term survey of tooth loss in 600 treated periodontal patients. J *Periodontol* 49:225-237.

Hujoel PP, DeRouen TA (1995). A survey of endpoint characteristics in periodontal clinical trials published 1988-1992, and implications for future studies. J *Clin Periodontol* 22:397-407.

Hujoel PP, Leroux BG, DeRouen TA, Powell LV, Kiyak HA (1997). Evaluating the validity of probing attachment loss as a surrogate for tooth mortality in a clinical trial on the elderly. J *Dent Res* 76:858-866.

Jones AA, Kornman KS, Newbold DA, Manwell MA (1994). Clinical and microbiological effects of controlled-release locally delivered minocycline in periodontitis. J *Periodontol* 65:1058-1066.

Joyston-Bechal S, Smales FC, Duckworth R (1984). Effect of metronidazole on chronic periodontal disease in subjects using a topically applied chlorhexidine gel. J *Clin Periodontol* 11:53-62.

Kaldahl WB, Kalkwarf PI, Patil KD (1993). A review of longitudinal studies that compared periodontal therapies. J *Periodontol* 64:243-253.

Kamma JJ, Nakou M, Manti FA (1994). Microbiota of rapidly progressive periodontitis lesions in association with clinical parameters. J *Periodontol* 65:1073-1078.

Keyes PH (1968). Research in dental caries. J *Am Dent Assoc* 76:1357-1373.

Keyes PH, Wright WE, Howard S (1978). The use of the phase contrast microscopy and chemotherapy in the diagnosis and treatment of periodontal lesions—initial report. *Quintessence Int Rep* 15:90-92.

Kulkarni GV, Lee WK, Aitken S, Birek P, McCulloch CA (1991). A randomized, placebo-controlled trial of doxycycline: effect on the microflora of recurrent periodontitis lesions in high risk patients. J *Periodontol* 62:197-202.

Lacroix JM, Walker CB (1995). Detection and incidence of the tetracycline resistance determinant tet (M) in the microflora associated with adult periodontitis. J *Periodontol* 66:102-108.

Larsen T (1991). Occurrence of doxycycline resistant bacteria in the oral cavity after local administration of doxycycline in patients with periodontal disease. *Scand J Infect Dis* 23:89-95.

Larsen T, Fiehn NE (1997). Development of resistance to metronidazole and minocycline in vitro. J *Clin Periodontol* 24:254-259.

Legg JA, Wilson M (1990). The prevalence of beta-lactamase producing bacteria in subgingival plaque and their sensitivity to Augmentin. Br J *Oral Maxillofac Surg* 28:180-184.

Levy D, Csima A, Birek P, Ellen RP, McCulloch CA (1993). Impact of microbiological consultation on clinical decision making: a case-control study of clinical management of recurrent periodontitis. J *Periodontol* 64:1029-1039.

Lindhe J (1982). Treatment of localized juvenile periodontitis. In: Host-parasite interactions in periodontal disease. Genco RJ, Mergenhagen SE, editors. Washington, DC: ASM Publications, pp. 382-394.

Lindhe J, Liljenberg B (1984). Treatment of localized juvenile periodontitis. Results after 5 years. J *Clin Periodontol* 11:399-410.

Lindhe J, Liljenberg B, Listgarten MA (1980). Some microbiological and histopathological features of periodontal disease in man. J *Periodontol* 51:264-269.

Lindhe J, Liljenberg B, Adielson B, Borjesson I (1983). Use of metronidazole as a probe in the study of human periodontal disease. J *Clin Periodontol* 10:100-112.

Listgarten MA (1992). Monitoring the periodontal microbiota as an adjunct to periodontal therapy: rationale, interpretation of test results and application to patient management. *Alpha Omegan* 85:49-53.

Listgarten MA, Helldén L (1978). Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. J *Clin Periodontol* 5:115-121.

Listgarten MA, Lai CH, Young V (1993). Microbial composition and pattern of antibiotic resistance in subgingival microbial samples from patients with refractory periodontitis. J *Periodontol* 64:155-161.

Loesche WJ (1976). Chemotherapy of dental plaque infections. *Oral Sci Rev* 9:65-107.

Loesche WJ (1993a). Dental caries: a treatable infection. Grand Haven, MI: Automated Diagnostic Documentation Inc., pp. 1-550.

Loesche WJ (1993b). Bacterial mediators in periodontal disease. Anaerobic bacteria and anaerobic infections. *Clin Infect Dis* 16(Suppl 4):S203-S210.

Loesche WJ (1994). Periodontal disease as a risk factor for heart disease. *Compend Cont Educ Dent* 15:976-991.

Loesche WJ, Giordano JR (1994). Metronidazole in periodontitis V: debridement should precede medication. *Compend Cont Educ Dent* 15:1198-1218.

Loesche WJ, Giordano JR (1997). Treatment paradigms in periodontal disease. *Compend Cont Educ Dent* 18:221-232.

Loesche WJ, Laughon B (1982). Role of spirochetes in periodontal disease. In: Host-parasite interactions in periodontal disease. Genco RJ, Mergenhagen SE, editors. Washington, DC: ASM Publications, pp. 62-80.

Loesche WJ, Lopatin DE (1998). Interactions between periodontal disease, medical diseases and immunity in the older individual. *Periodontol* 2000 16:80-105.

Loesche WJ, Nafe D (1973). Reduction of supragingival plaque accumulation in institutionalized Down's syndrome patients by periodic treatment with topical kanamycin. *Arch Oral Biol* 18:1131-1143.

Loesche WJ, Syed SA, Morrison EC, Kerry GA, Higgins T, Stoll J (1984). Metronidazole in periodontitis. I. Clinical and bacteriological results after 15 to 30 weeks. J *Periodontol* 55:325-335.

Loesche WJ, Syed SA, Schmidt E, Morrison EC (1985). Bacterial profiles of subgingival plaques in periodontitis. J *Periodontol* 56:447-456.

Loesche WJ, Bretz WA, Kerschensteiner D, Stoll JA, Socransky SS, Hujoel PP, *et al.* (1990). Development of a diagnostic test for anaerobic periodontal infections

based on plaque hydrolysis of benzoyl-DL-arginine-naphthylamide. J *Clin Microbiol* 28:1551-1559.

Loesche WJ, Schmidt E, Smith BA, Morrison EC, Caffesse R, Hujoel PP (1991). Effects of metronidazole on periodontal treatment needs. J *Periodontol* 62:247-257.

Loesche WJ, Giordano JR, Hujoel P, Schwarcz J, Smith BA (1992a). Metronidazole in periodontitis: reduced need for surgery. J *Clin Periodontol* 19:103-112.

Loesche WJ, Lopatin DE, Stoll J, van Poperin N, Hujoel PP (1992b). Comparison of various detection methods for periodontopathic bacteria: can culture be considered the primary reference standard? J *Clin Microbiol* 30:418-426.

Loesche WJ, Lopatin DE, Giordano J, Alcoforado G, Hujoel P (1992c). Comparison of the benzoyl-DL-arginine-naphthylamide (BANA) test, DNA probes, and immunological reagents for ability to detect anaerobic periodontal infections due to *Porphyromonas gingivalis*, *Treponema denticola*, and *Bacteroides forsythus*. J *Clin Microbiol* 30:427-433.

Loesche WJ, Grossman N, Giordano J (1993). Metronidazole in periodontitis (IV). The effect of patient compliance on treatment parameters. J *Clin Periodontol* 20:96-104.

Loesche WJ, Giordano J, Soehren S, Hutchinson R, Rau CF, Walsh L, *et al.* (1996). Nonsurgical treatment of patients with periodontal disease. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 81:533-543.

Loesche WJ, Kazor CE, Taylor GW (1997). The optimization of the BANA test as a screening instrument for gingivitis among subjects seeking treatment. J *Clin Periodontol* 24:718-726.

Loesche WJ, Schork A, Terpenning MS, Chen Y-M, Dominguez L, Grossman N (1998a). Assessing the relationship between dental disease and coronary heart disease in elderly US veterans. J *Am Dent Assoc* 129:301-311.

Loesche WJ, Schork MA, Terpenning MS, Chen Y-M, Kerr C, Dominguez L (1998b). The relationship between dental disease and cerebral vascular accident in elderly United States veterans. *Ann Periodontol* 3:161-174.

Lopez N, Gamonal JA (1998). Effects of metronidazole plus amoxicillin in progressive untreated adult periodontitis: report of a single 1-week course after 2 and 4 months. J *Periodontol* 69:1291-1298.

Lowenguth RA, Chin I, Caton JG, Cobb CM, Drisko CL, Killoy WJ (1995). Evaluation of periodontal treatments using controlled-release tetracycline fibers: microbiological response. J *Periodontol* 66:700-707.

Lundstrom A, Johansson LA, Hamp SE (1984). Effect of combined systemic antimicrobial therapy and mechanical plaque control in patients with recurrent periodontal disease. J *Clin Periodontol* 11:321-330.

MacDonald JB, Socransky SS, Gibbons RJ (1962). On the pathogenesis of mixed anaerobic infections of mucous membranes. *Ann R Coll Surg Engl* 31:361-378.

Magnusson I, Low SB, McArthur WP, Marks RG, Walker CB, Maruniak J, *et al.* (1994). Treatment of subjects with refractory periodontal disease [published *erratum* appears in J *Clin Periodontol* 22:183, 1995]. J *Clin Periodontol* 21:628-637.

Mahmood MM, Dolby AE (1987). The value of systemically administered metronidazole in the modified Widman flap procedure. J *Periodontol* 58:147-152.

Maiden MF, Tanner A, McArdle S, Najpauer K, Goodson JM (1991). Tetracycline fiber therapy monitored by DNA probe and cultural methods. J *Periodont Res* 26:452-459.

Mandel ID (1995). Calculus update: prevalence, pathogenicity and prevention. J *Am Dent Assoc* 126:573-580.

Mandell RL, Socransky SS (1988). Microbiological and clinical effects of surgery plus doxycycline on juvenile periodontitis. J *Periodontol* 59:373-379.

Mandell RL, Tripodi LS, Savitt E, Goodson JM, Socransky SS (1986). The effect of treatment on *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis. J *Periodontol* 57:94-99.

Mandell RL, Ebersole JL, Socransky SS (1987). Clinical, microbiological and immunological features of active disease sites in juvenile periodontitis. J *Clin Periodontol* 14:534-540.

Matthews DC, McCulloch CA (1993). Evaluating patient perceptions as short-term outcomes of periodontal treatment: a comparison of surgical and non-surgical therapy. J *Periodontol* 64:1029-1039.

Mattila KJ, Valtonen VV, Nieminen MS, Huttunen JK (1995). Dental infection and the risk of new coronary events: prospective study of patients with documented coronary artery disease. *Clin Infect Dis* 20:588-592.

Mattila KJ, Valtonen VV, Nieminen MS, Asikaninen S (1998). Role of infection as risk factors for atherosclerosis, myocardial infarction, and stroke. *Clin Infect Dis* 26:719-734.

McCulloch CA, Birek P, Overall C, Aitken S, Lee W, Kulkarni G (1990). Randomized controlled trial of doxycycline in prevention of recurrent periodontitis in high-risk patients: antimicrobial activity and collagenase inhibition. J *Clin Periodontol* 17:616-622.

McFall WT (1982). Tooth loss in 100 treated patients with periodontal disease. A long-term study. J *Periodontol* 53:539-549.

Meador HL, Lane JJ, Suddick RP (1985). The long-term effectiveness of periodontal therapy in a clinical practice. J *Periodontol* 56:253-258.

Mombelli A, Gusberti F, Lang NP (1989). Treatment of recurrent periodontal disease by root planing and Ornidazole. J *Clin Periodontol* 16:38-45.

Mombelli A, Tonetti M, Lehmann B, Lang NP (1996). Topographic distribution of black-pigmenting anaerobes before and after periodontal treatment by local delivery of tetracycline. J *Clin Periodontol* 23:906-913.

Moore WEC, Moore LVH (1994). The bacteria of periodontal diseases. *Periodontol* 2000 5:66-77.

Nagasawa M, Smith MC, Barnes JH, Fincham JE (1990). Meta-analysis of correlates of diabetes patients' compliance with prescribed medications. *Diabetes Educ* 16:192-200.

Newman MG, Socransky SS (1977). Predominant cultivable microbiota in periodontitis. J *Periodont Res* 12:120-127.

Newman MG, Kornman KS, Doherty FM (1994). A 6-month multi-center evaluation of adjunctive tetracycline fiber therapy used in conjunction with scaling and root planing in maintenance patients: clinical results. J *Periodontol* 65:685-691.

Novak MJ, Stamatelakys C, Adair SM (1991). Resolution of early lesions of juvenile periodontitis with tetracycline therapy alone: long-term observations of 4 cases. J *Periodontol* 62:628-633.

Offenbacher S, Katz VL, Fertik GS, Collins JG, Boyd DL, Maynor GB, *et al.* (1996). Periodontal infection as a possible risk factor for preterm low birth weight. J *Periodontol* 67:1103-1113.

Oldach DW, Calia FM (1998). Clindamycin. In: Infectious diseases. 2nd edition. Gorbach S, Bartlett J, Blacklow N, editors. Philadelphia: W.B. Saunders Co., pp. 232-242.

Olsvik B, Tenover FC (1993). Tetracycline resistance in periodontal pathogens. *Clin Infect Dis* 16(Suppl 4):S310-S313.

Pack PD, Haber J (1983). The incidence of clinical infection after periodontal surgery. A retrospective study. J *Periodontol* 54:441-443.

Palmer RM, Watts TL, Wilson RF (1996). A double-blind trial of tetracycline in the management of early onset periodontitis. J *Clin Periodontol* 23:670-674.

Papapanou PN, Baelum V, Luan WM, Madianos PN, Chen X, Fejerskov O, *et al.* (1997). Subgingival microbiota in adult Chinese: prevalence and relation to periodontal disease progression. J *Periodontol* 68:651-666.

Pavicic MJ, van Winkelhoff AJ, de Graaff J (1992). In vitro susceptibilities of *Actinobacillus actinomycetemcomitans* to a number of antimicrobial combinations. *Antimicrob Agents Chemother* 36:2634-2638.

Pihlstrom B (1992). Measurement of attachment level in clinical trial: probing methods. J *Periodontol* 63:1072-1077.

Polson AM, Garrett S, Stoller NH, Bandt CL, Hanes PJ, Killoy WJ, *et al.* (1997a). Multi-center comparative evaluation of subgingivally delivered sanguinarine and doxycycline in the treatment of periodontitis. I. Study design, procedures, and management. J *Periodontol* 68:110-118.

Polson AM, Garrett S, Stoller NH, Bandt CL, Hanes PJ, Killoy WJ, *et al.* (1997b). Multi-center comparative evaluation of subgingivally delivered sanguinarine and doxycycline in the treatment of periodontitis. II. Clinical results. J *Periodontol* 68:119-126.

Quirynen M, Bollen CM, Vandekerckhove BN, Dekeyser C, Papaioannou W, Eyssen H (1995). Full- vs. partial-mouth disinfection in the treatment of periodontal infections: short-term clinical and microbiological observations. J *Dent Res* 74:1459-1467.

Radvar M, Pourtaghi N, Kinane DF (1996). Comparison of 3 periodontal local antibiotic therapies in persistent periodontal pockets. J *Periodontol* 67:860-865.

Rams TE, Keyes PH, Wright WE, Howard SA (1984). Long-term effects of microbiologically modulated periodontal therapy on advanced adult periodontitis. J *Am Dent Assoc* 111:429-441.

Rams TE, Babalola OO, Slots J (1990). Subgingival occurrence of enteric rods, yeasts and staphylococci after systemic doxycycline therapy. *Oral Microbiol Immunol* 5:166-168.

Rams TE, Feik D, Listgarten MA, Slots J (1992). *Peptostreptococcus micros* in human periodontitis. *Oral Microbiol Immunol* 7:1-6.

Renvert S, Dahlén G, Wikström M (1996). Treatment of periodontal disease based on microbiological diagnosis. Relation between microbiological and clinical parameters during 5 years. J *Periodontol* 67:562-571.

Riviere GR, Smith KS, Carranza N Jr, Tzagaroulaki E, Kay SL, Dock M (1995). Subgingival distribution of *Treponema denticola*, *Treponema socranskii*, and pathogen-related oral spirochetes: prevalence and relationship to periodontal status of sampled sites. J *Periodontol* 66:829-837.

Roe FJC (1983). Toxicological evaluation of metronidazole with particular reference to carcinogenic, mutagenic and teratogenic potential. *Surgery* 93:158-166.

Rosebury T (1962). Microorganisms indigenous to man. New York: McGraw-Hill, pp. 1-380.

Rustia M, Shubik P (1972). Induction of lung tumors and malignant lymphoma in mice by metronidazole. J *Natl Cancer Inst* 48:721-727.

Saxén L, Asikainen S (1993). Metronidazole in the treatment of localized juvenile periodontitis. J *Clin Periodontol* 20:166-171.

Sefton AM, Maskell JP, Beighton D, Whiley A, Shain H, Foyle D, *et al.* (1996). Azithromycin in the treatment of periodontal disease. Effect on microbial flora. J *Clin Periodontol* 23:998-1003.

Shinn DLS (1962). Metronidazole in acute ulcerative gingivitis. *Lancet* 1:1191-1192.

Skapski H, Lehner T (1976). A crevicular washing method

for investigating immune components of crevicular fluid in man. J *Periodont Res* 11:19-24.
Slots J (1982). Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. J *Clin Microbiol* 15:606-609.
Slots J, Rosling BG (1983). Suppression of the periodontopathic microflora in localized juvenile periodontitis by systemic tetracycline. J *Clin Periodontol* 10:465-486.
Slots J, Feik D, Rams TE (1990a). *Actinobacillus actinomycetemcomitans* and *Bacteroides intermedius* in human periodontitis: age relationship and mutual association. J *Clin Periodontol* 17:659-662.
Slots J, Feik D, Rams TE (1990b). Prevalence and antimicrobial susceptibility of *Enterobacteriaceae*, *Pseudomonadaceae* and *Acinetobacter* in human periodontitis. *Oral Microbiol Immunol* 5:149-154.
Socransky SS, Haffajee AD (1993). Effect of therapy on periodontal infections. J *Periodontol* 64:754-759.
Socransky SS, Haffajee AD, Cugini MA, Smith CM, Kent RL Jr (1998). Microbial complexes in subgingival plaque. J *Clin Periodontol* 25:134-144.
Söder PÖ, Frithiof L, Wikner S, Wouters F, Engström P-E, *et al.* (1990). The effect of systemic metronidazole after non-surgical treatment in moderate and advanced periodontitis in young adults. J *Periodontol* 61:281-288.
Soskolne WA (1997). Subgingival delivery of therapeutic agents in the treatment of periodontal diseases. *Crit Rev Oral Biol Med* 8:164-174.
Soskolne WA, Heasman PA, Stabholz A, Smart GJ, Palmer J, Flashner M, *et al.* (1997). Sustained local delivery of chlorhexidine in the treatment of periodontitis: a multi-center study. J *Periodontol* 68:32-38.
Sterry KA, Langeroudi M, Dolby AE (1985). Metronidazole as an adjunct to periodontal therapy with sub-gingival curettage. *Br Dent* J 158:176-178.
Stewart RB, Caranasos GJ (1989). Medication compliance in the elderly. *Med Clin North Am* 73:1551-1563.
Stoltze K (1995). Elimination of Elyzol 25% Dentalgel matrix from periodontal pockets. J *Clin Periodontol* 22:185-187.
Syrjänen J (1990). Vascular diseases and oral infections. J *Clin Periodontol* 17:497-500.
Tally FP, Goldin BR, Sullivan N, Johnson J, Gorback SL (1978). Antimicrobial activity of metronidazole in anaerobic bacteria. *Antimicrob Agents Chemother* 13:460-465.
Tanner ACR, Haffer C, Bratthall GT, Visconti RA, Socransky SS (1979). A study of the bacteria associated with advancing periodontitis in man. J *Clin Periodontol* 6:278-307.
Timmerman MF, van der Weijden GA, van Steenbergen TJ, Mantel MS, de Graaff J, Van der Velden U (1996). Evaluation of the long-term efficacy and safety of locally-applied minocycline in adult periodontitis patients. J *Clin Periodontol* 23:707-716.
Tonetti MS, Cugini MA, Goodson JM (1990). Zero-order delivery with periodontal placement of tetracycline-loaded ethylene vinyl acetate fibers. J *Periodont Res* 25:243-249.
Topoll HH, Lange DE, Muller RF (1990). Multiple periodontal abscesses after systemic antibiotic therapy. J *Clin Periodontol* 17:268-272.
Tseng CC, Huang CC, Tseng WH (1993). Incidence of clinical infection after periodontal surgery: a prospective study. J *Formosa Med Assoc* 92:152-156.
Umeda M, Tominaga Y, He T, Yano K, Watanabe H, Ishikawa I (1996). Microbial flora in the acute phase of periodontitis and the effect of local administration of minocycline. J *Periodontol* 67:422-427.
Valtonen VV (1991). Infection as a risk factor for infarction and atherosclerosis. *Ann Med* 23:539-543.
van Steenberghe D, Bercy P, Kohl J, DeBoever J, Adriaens P, Vanderfaeillie A, *et al.* (1993). Subgingival minocycline hydrochloride ointment in moderate to severe chronic adult periodontitis: a randomized, double-blind, vehicle-controlled, multicenter study. J *Periodontol* 64:637-644.
van Winkelhoff AJ, Rodenburg JP, Goene RJ, Abbas F, Winkel EG, de Graaff J (1989). Metronidazole plus amoxycillin in the treatment of *Actinobacillus actinomycetemcomitans* associated periodontitis. J *Clin Periodontol* 16:128-131.
van Winkelhoff AJ, Tijhof CJ, de Graaff J (1992). Microbiological and clinical results of metronidazole plus amoxicillin therapy in *Actinobacillus actinomycetemcomitans*-associated periodontitis. J *Periodontol* 63:52-57.
van Winkelhoff AJ, Winkel EG, Barendregt D, Dellemijn-Kippuw N, Stijne A, Van der Velden U (1997). Beta-lactamase producing bacteria in adult periodontitis. J *Clin Periodontol* 24:538-543.
Vandekerckhove BN, Quirynen M, van Steenberghe D (1997). The use of tetracycline-containing controlled-release fibers in the treatment of refractory periodontitis. J *Periodontol* 68:353-361.
Wade WG, Moran J, Morgan JR, Newcombe R, Addy M (1992). The effects of antimicrobial acrylic strips on the subgingival microflora in chronic periodontitis. J *Clin Periodontol* 19:127-134.
Walker C, Gordon J (1990). The effect of clindamycin on the microbiota associated with refractory periodontitis. J *Periodontol* 61:692-698.
Walker CB, Pappas JD, Tyler KZ, Cohen S, Gordon JM (1985). Antibiotic susceptibilities of periodontal bacteria. In vitro susceptibilities to eight antimicrobial agents. J *Periodontol* 56:67-74.
Walker CB, Gordon JM, Magnusson I, Clark WB (1993). A role for antibiotics in the treatment of refractory periodontitis. J *Periodontol* 64:772-781.

Watts T, Palmer R, Floyd P (1986). Metronidazole: a double-blind trial in untreated human periodontal disease. J *Clin Periodontol* 13:939-943.

Winkel EG, van Winkelhoff AJ, Timmerman MF, Vangsted T, Van der Velden U (1997). Effects of metronidazole in patients with "refractory" periodontitis associated with *Bacteroides forsythus*. J *Clin Periodontol* 24:573-579.

Winkler JR, Murray PA, Grassi M, Hämmerle C (1989). Diagnosis and management of HIV-associated periodontal lesions. J *Am Dent Assoc* 120(Suppl):25S-34S.

Zambon JJ (1985). *Actinobacillus actinomycetemcomitans* in human periodontal disease. J *Clin Periodontol* 12:1-20.

Zambon JJ (1997). Rapid diagnosis of periodontal infections: findings in AIDS patients. *Immunol Invest* 26:55-65.