Systemic antibiotic therapy in periodontics
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CHAPTER 2

EFFECTS OF METRONIDAZOLE IN PATIENTS WITH "REFRACTORY" PERIODONTITIS ASSOCIATED WITH BACTEROIDES FORSYTHUS

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Abstract

The aim of the present study was to monitor the microbiological and clinical effects of renewed supra- and subgingival debridement in conjunction with systemic metronidazole therapy (500 mg TID for 7 days) in 27 "refractory" periodontitis patients, culture positive for Bacteroides forsythus and negative for Actinobacillus actinomycetemcomitans. Clinical evaluation included assessment of plaque, bleeding upon probing, probing pocket depth and clinical attachment loss at the deepest, bleeding site in each quadrant. Microbiological evaluation was carried out by anaerobic cultivation of subgingival plaque samples from the same sites. Six months after renewed debridement and systemic metronidazole (RD+M), a statistically significant improvement of all clinical parameters was observed, except for the plaque index. After RD+M, B. forsythus was suppressed below detection level in 17 of the 27 patients, P. gingivalis in 9 out of 15 patients and P. intermedia in 14 of the 21 patients. Before RD+M, 12 patients harboured simultaneously B. forsythus, P. gingivalis as well as P. intermedia. Out of these, 6 patients were culture negative for the 3 species after therapy and showed the greatest reduction in pocket depth (3.1 mm) and gain of clinical attachment level (2.5 mm). In the treatment of refractory periodontitis, associated with patients culture positive for B. forsythus and negative for A. actinomycetemcomitans, metronidazole can significantly improve the clinical and microbiological parameters.

Introduction

In practice, dentists are confronted with patients that respond poorly to conventional periodontal treatment as well as maintenance patients with recurrent disease activity despite proper oral hygiene care. We use the word "refractory" when conventional therapy does not result in a reasonable degree of healing. Factors responsible for refractory periodontitis may involve the composition of the subgingival microflora (Haffajee et al. 1988), impaired host defence (Genco & Mergenhagen 1979) and medical disorders (Schenkein & Van Dyke 1994), smoking (Haber et al. 1993, Martinez-Canut et al. 1995, Preber & Bergström 1986, Preber et al. 1995) and stress (Green et al. 1986). Up to 21% of the periodontitis patients may suffer from refractory periodontitis (Haffajee et al. 1988). Treatment possibilities in periodontal practice are restricted to oral hygiene measures, supra- and subgingival debridement, periodontal surgery and use of antibiotics. Subjects refractory to periodontal therapy, are prime candidates for continued conventional periodontal treatment in conjunction with systemic antimicrobial therapy (Slots 1996). Although the subgingival microflora in refractory periodontitis mainly consists of strict anaerobic bacteria, the facultative Gram-negative Actinobacillus actinomy-
cetemcomitans has been considered as one of the prime organisms (Van Winkelhoff et al. 1989, 1992, 1996).

Another bacterium implicated in the etiology of refractory periodontitis is Bacteroides forsythus, an anaerobic Gram-negative fusiform microorganism. In a series of studies by Socransky and co-workers, it was found that B. forsythus was a predominant organism in patients with the highest percentage of sites, showing loss of attachment after periodontal treatment (Dzink et al. 1988, Haffajee et al. 1988, Socransky et al. 1988a,b, 1994).

For the treatment of refractory periodontitis several antibiotics regimes have been advocated as adjuncts to mechanical periodontal therapy (Slots & Rams 1990, Van Winkelhoff et al. 1996). Studies by Van Winkelhoff et al. (1989, 1992) have shown that A. actinomycetemcomitans-associated refractory periodontitis can be successfully treated by adjunctive systemic use of the combination of amoxicillin and metronidazole. Other studies have documented the effects of metronidazole and ornidazole in refractory and recurrent periodontitis (Gusberti et al. 1988, Mombelli et al. 1989). However, in these studies the microbiological composition of the subgingival microflora was not a selection criterion.

The aim of this investigation was to monitor the microbiological and clinical effects of renewed debridement in conjunction with systemic metronidazole therapy in "refractory" periodontitis patients, culture-negative for A. actinomycetemcomitans but positive for B. forsythus.

Materials and Methods

Patients' group description

Patients in this study were selected from 2 private dental practices, both specialized in periodontics. All patients had a history of a routine initial periodontal therapy (RIPT) consisting of oral hygiene instructions and scaling and root planing. This treatment was carried out over a period of approximately 4 months and had amounted \( \geq 6 \) h. On evaluation, 3 months after the RIPT was completed, little or no pocket depth reduction and sustained high bleeding upon probing in comparison to values before RIPT were observed, despite improved oral hygiene. In this group of patients, diagnosed with refractory periodontitis, a subgingival plaque sample was taken from the deepest bleeding pocket in each quadrant. The samples of these 4 experimental sites were pooled. On the basis of the microbiological results, 27 subjects were selected showing subgingival presence of B. forsythus without detectable A. actinomycetemcomitans. Throughout the study these 4 experimental sites were used for clinical and microbiological analysis.
The mean age of the patients was 45 years and ranged from 29-64 years. The frequency distribution of probing pocket depth categories, based on 6 sites per tooth, of the 27 untreated periodontitis patients before RIPT are summarized in Table 1.

Table 1. Frequency of probing pocket depth (PD) categories of 27 untreated periodontitis patients.

<table>
<thead>
<tr>
<th>PD</th>
<th>Mean %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4</td>
<td>60.2</td>
<td>6.2-88.7</td>
</tr>
<tr>
<td>5</td>
<td>17.0</td>
<td>8.3-35.9</td>
</tr>
<tr>
<td>6-7</td>
<td>16.2</td>
<td>0.7-54.3</td>
</tr>
<tr>
<td>≥8</td>
<td>6.6</td>
<td>0.0-27.1</td>
</tr>
</tbody>
</table>

The proportion of sites with a probing pocket depth ≥ 6 mm was 22.8%. In retrospect, the mean probing pocket depth of the 4 experimental sites before the routine initial treatment was 6.9 ± 1.2 mm and amounted to 6.8 ± 0.9 mm when entering the study and also the bleeding on probing score remained virtually unchanged (0.9 ± 0.2 versus 0.9 ± 0.1). These observations confirm that the RIPT treatment has been ineffective in improving the periodontal condition which was the basis for further periodontal treatment.

Treatment

When entering the study, patients were subjected to renewed supra- and sub-gingival debridement for approximately 1 h after which they received a systemic metronidazole therapy of 500 mg TID for 7 days. A regular recall program was initiated. Six months later after renewed debridement plus metronidazole (RD+M), again at the 4 experimental sites, a re-examination was carried out to monitor the clinical and microbiological effects of RD+M therapy. Before and after RD+M the following clinical parameters were assessed at the 4 experimental sites.

Clinical measurements

1) Plaque index (Pll) of Sillness & Löe (1964) was used.
2) Probing pocket depth (PD) were assessed using a Hu-Friedy PQW probe.
3) Bleeding on probing (Bl) was scored as 0, no bleeding; 1, minor bleeding; and 2, immediate overt bleeding.
4) Clinical attachment level (CAL) was determined by subtraction of the distance between the gingival margin and the cemento-enamel junction from the recorded probing depth.
Microbiological procedures

After removal of supragingival plaque, a subgingival sample from the deepest bleeding site was taken in each quadrant, using 2 sterile paper points per site. All paper points were pooled and collected in reduced transport fluid (RTF) (Syed & Loesche 1972) and processed within 36 hours. Tenfold serial dilutions were prepared in RTF and aliquots of 0.1 ml were plated onto 5% horse blood agar plates supplemented with haemin (5 mg/l) and menadione (1 mg/l) for isolation and growth of obligately anaerobic bacteria, and on TSBV for selective isolation and growth of *A. actinomycetemcomitans* (Slots 1982). Blood agar plates were incubated anaerobically in 80% N₂, 10% H₂ and 10% CO₂ for up to 14 days and TSBV plates were incubated in air + 5% CO₂ for 5 days (Van Steenbergen et al. 1986). Blood agar plates were used for enumeration of dark-pigmented colonies and *B. forsythus*. Representative dark-pigmented colonies were purified and identified using standard techniques (Van Winkelhoff et al. 1985), including Gram-stain, hemagglutination of 3% sheep erythrocytes, fermentation of carbohydrates, production of indole from tryptophan and production of specific enzymes (Van Winkelhoff et al. 1986). *B. forsythus* was identified on the basis of the typical colony morphology, Gram-stain and production of a trypsinlike enzyme (Braham & Moncla 1992).

Statistical analyses

For statistical analyses, the BMDP/PC90 package was used. For analyses of the clinical data a patient level response variable was calculated for each parameter by computing the mean value of the scores assessed at the 4 experimental sites at each assessment. Differences in clinical parameters before and after RD+M therapy were analyzed using a Wilcoxon-test. To investigate effects of different microbiological treatment outcomes on the clinical response an analysis of variance and covariance with repeated measures was used, entering clinical parameters as dependent variables and plaque scores before and after RD+M as covariates. This analysis demonstrated differences in treatment response apart from those dependent on differences in the change of plaque scores. Values of $p < 0.05$ were accepted as statistically significant.
Results

The mean clinical parameters of the 4 experimental sites before and after RD+M are shown in Table 2.

Table 2. Mean clinical parameters (standard deviation) at the 4 experimental sites, before and after renewed debridement plus metronidazole therapy (RD+M) in 27 refractory periodontitis patients.

<table>
<thead>
<tr>
<th></th>
<th>Before RD+M</th>
<th>After RD+M</th>
<th>Change</th>
<th>p-value Wilcoxon</th>
<th>*p-value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PII</td>
<td>0.5 (0.6)</td>
<td>0.3 (0.5)</td>
<td>-0.2</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>BI</td>
<td>1.6 (0.5)</td>
<td>0.7 (0.4)</td>
<td>-0.9</td>
<td>0.0001</td>
<td>0.0000</td>
</tr>
<tr>
<td>PD</td>
<td>6.8 (1.0)</td>
<td>5.3 (1.2)</td>
<td>-1.5</td>
<td>0.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>CAL</td>
<td>7.7 (1.6)</td>
<td>6.7 (1.6)</td>
<td>-1.0</td>
<td>0.0002</td>
<td>0.007</td>
</tr>
</tbody>
</table>

PII: plaque index; BI: bleeding index; PD: probing pocket depth; CAL: clinical attachment level.
*p-values with plaque scores as a covariate.

With the exception for the plaque index, a statistically significant improvement of all clinical parameters was noted after the RD+M therapy. When the changes in clinical parameters were statistically tested with supragingival plaque scores as a co-variate, a significant change was still observed for the BI, PD and the CAL. The frequency distribution of probing pocket depth before and after RD+M therapy showed a shift toward shallower pocket depth (Fig. 1). The % of pockets ≥ 6 mm decreased from 80% before RD+M to 36% after RD+M. The same trend was observed in relation with the frequency distribution of the attachment level measurements (Fig. 2).

Table 3 summarises the frequency of isolation of Bacteroides forsythus, Porphyromonas gingivalis and Prevotella intermedia and the mean % and the median values before and after RD+M therapy. Before RD+M all 27 patients had detectable subgingival B. forsythus. After RD+M in 17 patients B. forsythus was no longer detectable, a reduction of 63% P. gingivalis was present in 15 of 27 patients. In 9 of the 15 subjects P. gingivalis was suppressed below detection level. Of the 12 initially P. gingivalis negative patients, 5 had detectable levels of this bacterium after therapy (range 1.6%-15.9%). Out of these, 1 patient (P. gingivalis = 10.5%) showed an increase (1.0 mm ) and 3 subjects a decrease in pocket depth (mean 0.9 mm, range 0.5-1.3 mm). In 1 patient no change in PD was observed. The 2 patients showing the highest levels of P. gingivalis (10.5%,15.9%) had the highest plaque scores of the entire study population after RD+M therapy.

Fourteen of the 21 patients became negative for P. intermedia. Of the 6 patients initially negative for P. intermedia, 3 patients became positive (range 0.1%-3.4%).
Fig. 1. Frequency distribution of **probing pocket depth** in 27 refractory periodontitis patients before and after renewed debridement plus metronidazole (RD+M) at 4 experimental sites.

- **Black** : Before RD+M
- **Light Gray** : After RD+M

Fig. 2. Frequency distribution of **attachment level** in 27 refractory periodontitis patients before and after renewed debridement plus metronidazole (RD+M) at 4 experimental sites.

- **Black** : Before RD+M
- **Light Gray** : After RD+M
Table 3. Effects on selected bacterial species in % of the anaerobe cultivable microflora (standard deviation) in 27 periodontitis patients, before and after renewed debridement plus metronidazole (RD+M) at the 4 experimental sites.

<table>
<thead>
<tr>
<th>before RD+M</th>
<th>After RD+M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Bf positive</td>
<td>27</td>
</tr>
<tr>
<td>Pg positive</td>
<td>15</td>
</tr>
<tr>
<td>Pg negative</td>
<td>12</td>
</tr>
<tr>
<td>Pi positive</td>
<td>21</td>
</tr>
<tr>
<td>Pi negative</td>
<td>6</td>
</tr>
</tbody>
</table>

n: number of patients; ND: not detected; RD+M: renewed debridement plus metronidazole; SD: standard deviation.

Bf: Bacteroides forsythus; Pg: Porphyromonas gingivalis; Pi: Prevotella intermedia.
Table 4. Change of clinical parameters of 4 different groups, classified on the basis of the presence and/or absence of specific anaerobic cultivable microflora, before renewed debridement plus metronidazole (RD+M).

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>PII</th>
<th>BI</th>
<th>PD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before (SD)</td>
<td>after (SD)</td>
<td>change</td>
<td>before (SD)</td>
<td>after (SD)</td>
</tr>
<tr>
<td>+ + + +</td>
<td>0.7 (0.6)</td>
<td>0.3 (0.3)</td>
<td>-0.4</td>
<td>1.6 (0.5)</td>
<td>0.6 (0.4)</td>
</tr>
<tr>
<td>+ + -</td>
<td>0.0 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.1</td>
<td>1.6 (0.7)</td>
<td>0.7 (0.6)</td>
</tr>
<tr>
<td>+ - +</td>
<td>0.4 (0.5)</td>
<td>0.4 (0.7)</td>
<td>0.0</td>
<td>1.6 (0.4)</td>
<td>0.7 (0.5)</td>
</tr>
<tr>
<td>+ - -</td>
<td>0.3 (0.3)</td>
<td>0.2 (0.3)</td>
<td>-0.1</td>
<td>1.6 (0.5)</td>
<td>0.4 (0.1)</td>
</tr>
</tbody>
</table>

n: number of patients; PII: plaque index; BI: bleeding index; PD: probing pocket depth; CAL: clinical attachment level; SD: standard deviation.

BF: Bacteroides forsythus; PG: Porphyromonas gingivalis; PI: Prevotella intermedia.

* Wilcoxon: change of clinical parameters before and after RD+M, p < 0.05.

** Wilcoxon: change of clinical parameters before and after RD+M, p < 0.005.
Table 5. Change of clinical parameters of 3 different groups, classified on the basis of presence of specific anaerobe cultivable microflora before and after RD+M.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before RD+M</th>
<th>After RD+M</th>
<th>Change PI</th>
<th>Change BI</th>
<th>Change PD</th>
<th>Change CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bf</td>
<td>Pg</td>
<td>Pi</td>
<td>Bf</td>
<td>Pg</td>
<td>Pi</td>
</tr>
<tr>
<td>1 a</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b</td>
<td>17</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 a</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3 a</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n: number of patients; RD+M: renewed debridement plus metronidazole; PI: plaque index; BI: bleeding index; PD: probing pocket depth; CAL: clinical attachment level; SD: standard deviation.

Bf: Bacteroides forsythus; Pg: Porphyromonas gingivalis; Pi: Prevotella intermedia.

* ANOVA: plaque scores as a covariate. Change of clinical parameters before and after RD+M, p < 0.05.

** ANOVA: plaque scores as a covariate. Change of clinical parameters before and after RD+M, p < 0.005.
All 3 patients had a decrease in mean PD ≥ 1.0 mm. Of the 5 patients who had an increase of the plaque score after RD+M, 4 became positive for *P. gingivalis* and/or *P. intermedia*.

None of the patients had detectable levels of *A. actinomycetemcomitans* at any examination.

If a selection of patients was made on the basis of presence or absence of *B. forsythus, P. gingivalis* and *P. intermedia* before RD+M, the reduction of probing pocket depths and the gain of clinical attachment was most pronounced in subjects positive for all 3 periodontal pathogens before RD+M (Table 4). In this group, a reduction of pocket depth of 2.1 mm and a gain of clinical attachment of 1.4 mm was observed. In patients initially positive for *B. forsythus* but without detectable *P. gingivalis* and/or *P. intermedia* a less favourable clinical result was noted.

Table 5 summarises a further analysis of the relationship between clinical parameters and the presence of selected periodontal microorganisms. Three different groups can be recognized on the basis of the combination of the microbiological data before and after RD+M therapy. Group 1 represents the patients in relation to the presence or absence of *B. forsythus*, irrespective of the results for *P. gingivalis* and *P. intermedia*. In group 1a, representing the patients positive for *B. forsythus* post-treatment (n=10), the only clinical change was reduction in bleeding on probing. In 17 of 27 patients, *B. forsythus* was no longer detectable after RD+M therapy (group 1b) which was paralleled by an improvement of all clinical parameters. When the absence of *P. gingivalis* was taken into account, a mean reduction in PD of 2.6 mm and mean gain of CAL of 2.1 mm was observed (group 2b). The maximum reduction in PD (mean 3.1 mm) and CAL (mean 2.5 mm) was observed in group 3b which was marked by the presence of *B. forsythus, P. gingivalis* and *P. intermedia* before RD+M and the absence of these microorganisms after RD+M.

**Discussion**

The results of this study have shown that renewed debridement followed by systemic metronidazole can improve the clinical periodontal status of patients poorly responding to previous periodontal therapy.

In general most studies evaluating the additional effect of metronidazole in the treatment of periodontal disease, involve patients with adult periodontitis. As far as we are aware, only 2 studies have investigated the use of metronidazole in refractory/recurrent periodontitis. Gusberti *et al.* (1988), who treated 5 refractory periodontitis patients with 250 mg TID for 10 days and Mombelli *et al.* (1989), who used 500 mg ornidazole 2x daily for 10 days in 10 subjects with recurrent periodontitis, obtained comparable clinical results as we observed in the present study.
Metronidazole and *B. forsythus*

Apparently, in the present study a higher dose of metronidazole for a shorter period of time results in a similar treatment outcome. The question remains, whether renewed debridement without metronidazole would have resulted in the same clinical improvement as we found in the present study. It has been shown that the maximum clinical effect of mechanical debridement is obtained within a period of 6 months and retreatment does not result in further clinical improvement (Badersten et al. 1984, Claffey et al. 1988, 1991, 1994, 1995). Therefore, in this study we assume that it is unlikely that renewed debridement alone is responsible for the improvement of the clinical condition.

It is well-known that plaque control is of paramount importance in the treatment of periodontal disease. In the present study no significant plaque reduction was found when the analysis was carried out on all 27 subjects. However, a significant decrease of plaque was observed in the 12 patients positive for all 3 periodontal pathogens before renewed debridement plus metronidazole (Table 4). Our observations do not elucidate the question whether supragingival plaque prevents suppression of *B. forsythus, P. gingivalis* and *P. intermedia* or vice-versa, whether presence of subgingival *B. forsythus, P. gingivalis* and *P. intermedia* stimulates supragingival plaque growth. Ramberg et al. (1995, 1996) demonstrated that the rate of plaque formation adjacent to an inflamed gingiva was greater than to a healthy gingival segment. In this respect it is interesting to note that 4 of the 5 patients who showed an increase of the plaque score after RD+M, became positive for *P. gingivalis* and/or *P. intermedia*. It may be speculated that the reduction of the inflammation due to the decrease of periodontal pathogens leads to a decline of the crevicular fluid (Giedrys-Leeper 1985) and consequently a reduction of de novo plaque formation (Hillam & Hull 1977). In order to enhance the effect of systemic antibiotic therapies, every effort should be made to control the supragingival plaque (Kornman et al. 1994). Therefore, it may be justified to use chlorhexidine mouth rinses and/or gels simultaneously with systemic metronidazole (Joyston-Bechal et al. 1984).

The finding that in spite of the treatment some patients became positive for *P. gingivalis* and *P. intermedia* can be explained in the following way. Most likely the bacteria were already present before renewed debridement but were missed in the microbiological evaluation due to the lack of reproducibility of the sampling procedure (Dahldén et al. 1990) and/or presence of the target bacteria under detection level. After therapy, in some patients the numbers of bacteria may have increased due to higher levels of plaque post-therapy.

In retrospect, the results of the present study show that the best clinical improvement, in terms of reduction in pocket depth and gain of clinical attachment level, can be expected in patients who were at the start of the study simultaneously infected with *B. forsythus, P. gingivalis* and *P. intermedia*. This is in accordance with the
study of Haffajee et al. (1996) who also found a maximum clinical effect in patients with multiple putative pathogens at baseline. However in the present study, an even more pronounced effect was found in the patients who became negative for the 3 species after therapy. Therefore it is plausible to state that suppression of *B. forsythus*, *P. gingivalis* and *P. intermedia* below detection level significantly contributes to the clinical outcome of the treatment. Our data support the concept that the absence of these bacteria may be considered an endpoint of active periodontal treatment in susceptible individuals (Renvert et al. 1990) and a reliable predictor of no further loss of attachment (Wennström et al. 1987, Rams et al. 1996, Dahlén et al. 1996).

In conclusion, in the treatment of "refractory" periodontitis by means of renewed debridement plus systemic metronidazole, the most favourable clinical results can be expected in patients positive for *B. forsythus*, *P. gingivalis* and *P. intermedia* before and negative for these species after therapy.

References


Metronidazole and B. forsythus


Chapter 2


