Microbiological Outcomes of Systemic Ornidazole Use in Chronic Periodontitis. Part II

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Abstract

Objective: Antimicrobials are increasingly being used as adjuncts to non-surgical or surgical periodontal therapy. The main purpose of the present analysis was to evaluate the effect of systemic ornidazole (ORN) on total anaerobic microbial counts of subjects with moderate to advanced chronic periodontitis (CP). **Methods:** This was a single-center, double-blinded, placebo-controlled, randomized clinical trial of six months duration. Fifty-eight subjects presenting with at least 12 teeth with probing depth (PD) $\geqslant$ 4 mm were selected. Thirty subjects received full-mouth scaling and root planing (SRP) + placebo (control group) and 28 subjects received full-mouth SRP + ORN (test group). The total anaerobic counts were analyzed by collecting subgingival plaque from deepest pockets at baseline (B/L), 1 week, 1 month, 3 months and 6 months. **Results:** Paired and unpaired *t*-tests were used to determine the inter- and intra-group differences. Fifty subjects were evaluated up to six months. There was a significant difference in the number of anaerobes in the two groups at all the intervals except B/L ($p < 0.05$). **Conclusion:** The systemic use of ORN very efficiently reduced the microbial load in the group that received antibiotics.

Key words: Antimicrobials, periodontitis, scaling and root planing, statistics

Introduction

The main target of periodontal therapy is to achieve the clinical signs of a healthy periodontium, which includes the absence of inflammatory signs of disease such as redness, swelling, suppuration and bleeding on probing, and maintenance of a functional periodontal attachment level (American Academy of Periodontology, 2001). The treatment plan for periodontal diseases has a certain set of accepted protocols (American Academy of Periodontology, 2001). This begins with patient education and motivation. Following this, non-surgical periodontal treatment (NSPT) comprising supra- and subgingival scaling and root planing (SRP) is employed. Addition of chemotherapeutic agents to NSPT as well as any further treatment is well proven and widely accepted to reduce, eliminate or alter the microbial pathogens. The subgingival microbial profile of severe chronic periodontitis (CP) patients is highly diverse (Colombo *et al.*, 2009; Armitage, 2010).

In many clinical trials successful attempts have been made to reduce the microbial pathogens by using antimicrobial agents as adjuncts to NSPT (Kamma *et al.*, 2000; Cionca *et al.*, 2010; Rooney *et al.*, 2002; Winkel *et al.*, 1999; Winkel *et al.*, 2001; Pradeep and Kathariya, 2011). Ornidazole (ORN) is an antimicrobial agent belonging to the 5-nitroimidazole group. It is highly effective against anaerobes (Wust, 1978). The first report of successful use of metronidazole (MET) dates back to 1962, when it was used in the treatment of acute ulcerative gingivitis (Shinn *et al.*, 1965). Since then it has been used in the management of several anaerobic bacteria, including *Bacteroides fragilis, Clostridium difficile and Helicobacter pylori*. Despite 45 years of extensive use, MET remains the criterion standard for the management and prophylaxis of anaerobic infections. ORN has anti-bacterial properties comparable to or better than MET (Wust, 1978).

We recently reported the clinical results of a trial of six months duration on this same patient population (Pradeep *et al.*, 2012). Twenty-eight subjects in the test group received 500 mg ORN tablets while 30 control subjects received 500 mg placebo tablets. Significant differences in clinical parameters such as probing depth (PD) and clinical attachment level (CAL) were reported at different intervals. Microbiological counts were also analyzed at all the time intervals. The present paper reports the effects of ORN on total anaerobic

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microbial counts in the two groups of the previously conducted trial.

Materials and methods

Subject population

This was a six-month, single-center, placebo-controlled, randomized clinical trial. It was conducted on 58 subjects with a history of CP referred to the Department of Periodontics, Government Dental College and Research Institute (GDCRI), Bangalore, between November 2010 and July 2011. The study protocol was approved by the institutional ethical committee and review board, GDCRI, Bangalore, India. Written informed consent was obtained from all subjects before beginning the trial.

Inclusion and exclusion criteria

Fifty-eight systemically healthy subjects with untreated moderate to advanced periodontitis were recruited into the study based on the following criteria: age 25 to 60 years, the presence of at least 12 scorable teeth (not including third molars and teeth with orthodontic appliances, bridges, crowns, or implants) with a PD $\geqslant 4$ mm and radiographic evidence of bone loss.

Exclusion criteria included pregnancy or lactation, smoking, systemic diseases such as diabetes mellitus, systemic antibiotics taken within the previous two months, use of non-steroidal anti-inflammatory drugs, confirmed or suspected intolerance to 5-nitroimidazole derivatives, and subgingival SRP or surgical periodontal therapy in the previous year.

Treatment protocol

A detailed description of the treatment protocol was presented in a preceding article (Pradeep *et al.*, 2012). Briefly, 58 subjects were randomly assigned to one of the two treatments. Supragingival scaling (ultrasonic scaler, Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, China) was performed on all subjects by the operator (NK) one week before the baseline (B/L) visit. Strict oral hygiene instructions were given to all subjects at the same time by the operator/clinical examiner (NK). Subjects were also instructed to use 0.2% chlorhexidine (CHX) rinse twice daily during this period. Oral hygiene instructions were checked and reinforced at each point. One session of SRP was performed in all subjects at every interval.

At the B/L visit, the clinical parameters mentioned earlier were recorded in four teeth with the most severe destruction, both clinically and radiographically, at six sites/tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-palatal/lingual, mid-palatal/lingual and disto palatal/lingual) in each patient of both groups by the same person (NK). Next, the operator treated the periodontally diseased sites with thorough SRP (Gracey curettes, Hu-Friedy, Chicago, IL, USA) to the depth of the pocket under local anesthesia. After this each subject received a package containing the test or placebo medication; all packages were identical in appearance and were marked only with the subject number. Subjects in the test group received ORN (Giro 500 mg tablets, Panacea-Biotec, New Delhi, India.) to be taken twice daily for seven days (Kamma *et al.*, 2000); subjects in the control group received similarly looking placebos prepared at Government College of Pharmacy, Bangalore. Randomization and distribution of packets containing the tablets was done by the chief investigator (ARP). The treatment group was concealed from the patient, clinical examiner/operator, and statistician.

Microbiological monitoring

The sampling area was isolated with cotton rolls, carefully cleaned with sterile cotton pellets, and then air-dried. Subgingival plaque samples were collected from the periodontal sites with the deepest pockets with the help of sterile curettes (Gracey curette, Hu-Friedy) (Tanner and Goodson, 1986). A fixed reference marking was made on the curette, up to which the amount of plaque collected was sent for microbiological analysis. The samples were immediately transported to the lab in sterile bottles containing Robertson cooked meat broth (RCMB) (Sandhya *et al.*, 2011; Saini *et al.*, 2003). The samples were transported to the laboratory immediately.

The subgingival plaque samples were primarily cultured in RCMB and incubated in anaerobic jars for 48 hours at 37°C. Then they were sub-cultured on neomycin blood agar supplemented with vitamin K and hemin followed by incubation in an anaerobic jar at 37°C and the growth of organisms was analyzed each day for seven days. The combined counts of anaerobes were recorded at B/L, 1 week, 1 month, 3 months and 6 months.

Statistical analysis

Unpaired *t*-tests were used for comparing differences in total anaerobic count between the two groups. To compare the differences in number of anaerobes within each group, paired *t*-tests were used. The level of significance (α) was taken as 0.05 and the confidence intervals (CI) were set at 95%. Statistically significant differences were assumed at $p < 0.05$. Data analysis was performed using a commercially available statistical package (SPSS version 10; SPSS Inc., Chicago, IL, USA).

Results

A consort flowchart (*Figure 1*) describes the number of subjects analyzed and those who dropped out of the study.

Table 1 shows mean ± SD, *p* values and 95% confidence intervals (CI) of the differences between the two groups. There was a significant difference in the

Table 1. Mean ± SD, *p* values and 95% CI of differences between the total anaerobic counts of the two groups at various intervals

Parameter	Interval	Control (n = 25)	Test (n = 25)	*p*-value	95% CI	
					Lower	Upper
Microbiological count x 10^5 (mean ± SD)	B/L	87.28 ± 6.97	87.56 ± 15.09	0.933	-6.604	6.694
	1 week	57.48 ± 7.68	47.84 ± 13.64	0.003*	-15.935	-3.345
	1 month	51.72 ± 6.73	43.84 ± 10.22	0.002*	-12.800	-2.960
	3 months	61.16 ± 5.10	51.26 ± 9.39	0.001*	-14.197	-5.603
	6 months	68.56 ± 5.89	58.40 ± 9.82	0.001*	-14.764	-5.556

*Denotes statistically significant difference. CI, confidence interval; SD, standard deviation

Table 2. Comparison of microbial count (x 10^5) between baseline and other time intervals in ornidazole (ORN) group

Time interval	Mean ± SD	Mean difference	*t*-test value	*p*-value
Baseline	87.56 ± 15.09	39.72	16.883	< 0.001*
1 Week	47.84 ± 13.64			
Baseline	87.56 ± 15.09	43.72	20.373	< 0.001*
1 Month	43.84 ± 10.22			
Baseline	87.56 ± 15.09	36.30	14.555	< 0.001*
3 Months	51.26 ± 9.39			
Baseline	87.56 ± 15.09	29.16	11.366	< 0.001*
6 Months	58.4 ± 9.82			

*Denotes statistically significant difference. SD, standard deviation

viable anaerobic count at 1 week, 1 month, 3 months and 6 months ($p < 0.05$). At B/L the inter-group difference was not significant ($p > 0.05$). At week 1, the total anaerobic counts were 57.48 ± 7.68 x 10^5 and 47.84 ± 13.64 x 10^5 in placebo and ORN groups, respectively, with a *p* value of 0.003. At 1 month the total anaerobic counts dropped further to 51.72 ± 6.73 x 10^5 and 43.84 ± 10.22 x 10^5 ($p = 0.002$). At the end of the trial, a slight increase in the total anaerobic count was found in both the groups; the difference, however, was statistically significant ($p = 0.001$).

Table 2 demonstrates intra-group comparisons of assessed total anaerobic counts from B/L to 1 week, 1 month, 3 months and 6 months, which were found to be highly significant ($p < 0.001$) in both the control and ORN groups.

Discussion

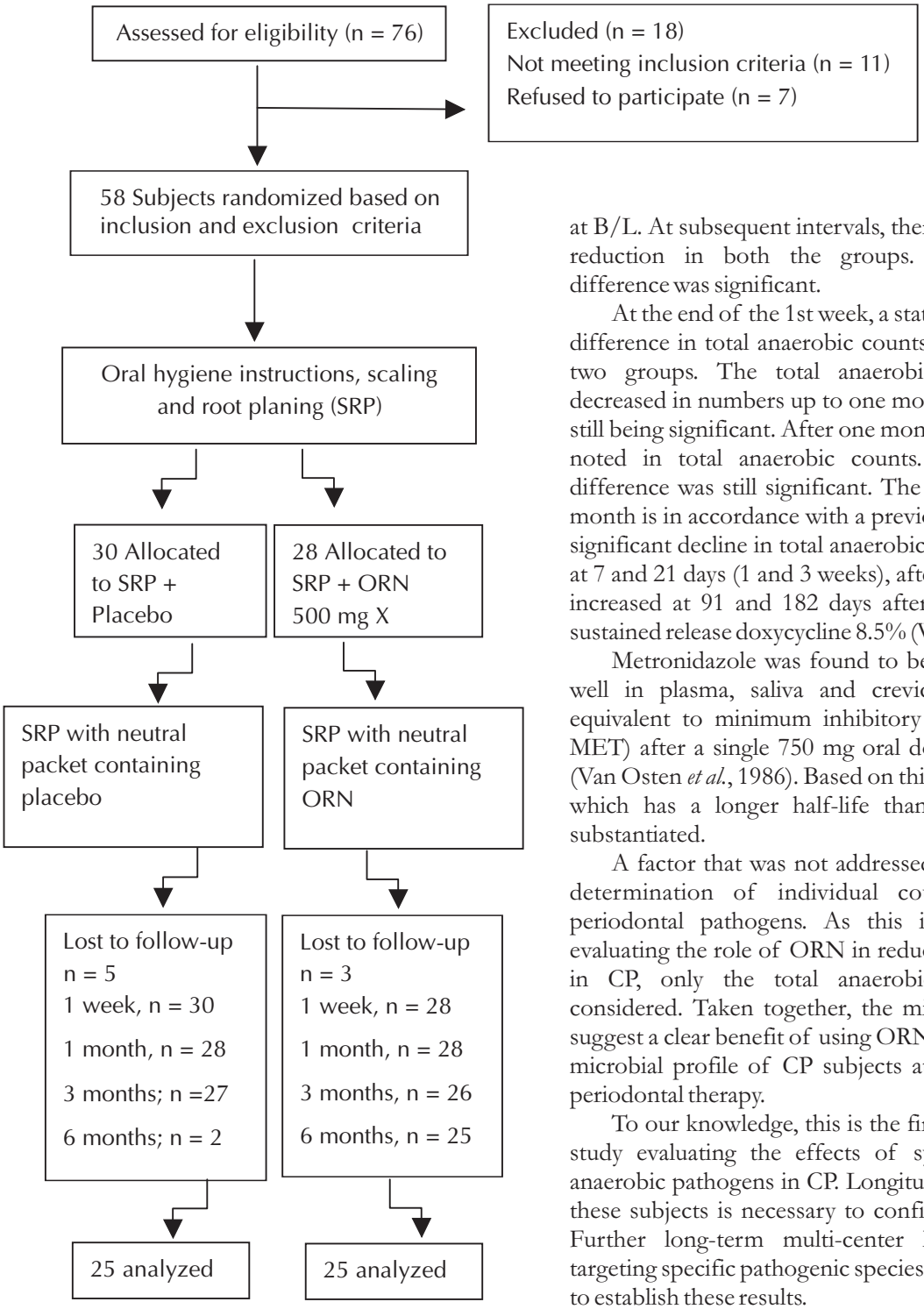
The primary aim of the present analysis is to determine whether or not systemic ORN used as an adjunct to NSPT in CP reduces the anaerobic count. ORN was found to be clearly beneficial in reducing the anaerobic bacterial load in CP subjects.

The rationale behind prescribing ORN as an adjunct to NSPT in the treatment of CP lies in the fact that the sub-gingival microbial profile of CP subjects is highly diverse, consisting of a large number of obligate anaerobes (Uematsu and Hoshino, 1992; Wennstrom *et al.*, 1987). As mentioned in our preceding article, the mean depths of periodontal pockets assessed were quite deep and anaerobic bacteria predominate in very deep pockets because of low oxygen tension (Loesche *et al.*, 1983, Mettraux *et al.*, 1984).

The adjunctive use of antibiotics such as MET, an antimicrobial agent specific for anaerobic bacteria, has been proved to be effective in eradicating or markedly reducing periodontal pathogens. ORN belongs to the 5-nitroimidazole group. It has a proven efficacy in reducing the viable anaerobic count (Cionca *et al.*, 2010; Rooney *et al.*, 2002; Winkel *et al.*, 2001). ORN was selected based on an earlier study utilizing ORN in the treatment of early onset periodontitis (Kamma *et al.*, 2000).

Most studies that evaluated the effectiveness of antimicrobial agents as adjunct to NSPT in CP have considered the specific periodontal pathogen count. In this trial, however, we have evaluated the total viable

Figure 1: Consort flowchart



anaerobic count. Our methodology is similar to a previous study by Sandhya YP *et al.*, 2003, in which total anaerobic colony counts were evaluated after local delivery of 10% doxycycline hyclate in smokers with periodontitis (Saini *et al.*, 2003). In yet another study, the total cultivable bacteria were counted after both local and systemic administration of minocycline in adult periodontitis subjects (Preus *et al.*, 1995).

Both groups exhibited similar levels of pathogens at B/L. At subsequent intervals, there was a significant reduction in both the groups. The inter-group difference was significant.

At the end of the 1st week, a statistically significant difference in total anaerobic counts was found in the two groups. The total anaerobic counts further decreased in numbers up to one month, the difference still being significant. After one month, an increase was noted in total anaerobic counts. The inter-group difference was still significant. The increase after one month is in accordance with a previous trial in which a significant decline in total anaerobic counts was found at 7 and 21 days (1 and 3 weeks), after which they were increased at 91 and 182 days after local delivery of sustained release doxycycline 8.5% (Walker *et al.*, 2000).

Metronidazole was found to be absorbed equally well in plasma, saliva and crevicular fluid (levels equivalent to minimum inhibitory concentration of MET) after a single 750 mg oral dose administration (Van Osten *et al.*, 1986). Based on this, the use of ORN, which has a longer half-life than MET, could be substantiated.

A factor that was not addressed in our study was determination of individual counts of specific periodontal pathogens. As this is the first study evaluating the role of ORN in reducing the anaerobes in CP, only the total anaerobic numbers were considered. Taken together, the microbiological data suggest a clear benefit of using ORN in the subgingival microbial profile of CP subjects at six months after periodontal therapy.

To our knowledge, this is the first microbiological study evaluating the effects of systemic ORN on anaerobic pathogens in CP. Longitudinal follow-up of these subjects is necessary to confirm these findings. Further long-term multi-center longitudinal trials targeting specific pathogenic species may be carried out to establish these results.

This study has shown that the systemic use of ORN, when used in conjunction with initial periodontal treatment consisting of SRP in adult subjects with periodontitis, achieves significantly better clinical results than initial periodontal treatment alone. A significant advantage of using anti-microbials as adjuncts to NSPT is that they reduce the need for further treatment, which could mean periodontal surgery, in turn reducing the cost associated with the treatment. These antimicrobials however, cannot be

used as a substitute to proper professional and thorough mechanical debridement and professional oral health education, which also produced excellent results.

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