A Randomized Controlled Clinical Trial on the Clinical and Microbiological Efficacy of Systemic Satranidazole in the Treatment of Chronic Periodontitis

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ABSTRACT

Objective: The present clinical trial was designed to investigate the effectiveness of systemic satranidazole (SZ) as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis. **Methods:** Sixty-six subjects presenting with at least twelve teeth with probing depth (PD) ⩾ 4 mm were selected. Thirty-three subjects were randomly assigned to full-mouth SRP + placebo (Group 1) and 33 subjects were assigned to full-mouth SRP + SZ (Group 2). The clinical outcomes evaluated were plaque index (PI), gingival index (GI), clinical attachment level (CAL) and PD at baseline, 1 month, 3 months and 6 months. Also, microbial analysis of dental plaque using polymerase chain reaction was done at baseline, 3 and 6 months to estimate the number of sites harboring periodontopathogens. **Results:** Sixty subjects were evaluated up to 6 months. At 6 months, Group 2 showed greater mean reduction (3.84 ± 1.31 mm) in PD as compared to Group 1 (1.42 ± 1.01 mm; $p < 0.05$) and there was a greater mean CAL gain (3.22 ± 1.01 mm) in Group 2 as compared to Group 1 (1.15 ± 1.49 mm; $p < 0.05$). These subjects also showed significant reductions in the number of certain periodontopathogens, such as *Tannerella forsythia, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans*. **Conclusion:** The systemic use of SZ, when used as an adjunct to non-surgical periodontal therapy in subjects with periodontitis, achieves significantly better clinical and microbiological results than scaling and root planing alone.

Key words: Satranidazole, chronic periodontitis, systemic antimicrobials, microbiology

Introduction

Chronic periodontitis (CP) is an infectious disease of the supporting tissues of the teeth. Because of bacterial infection, the periodontal tissues become inflamed and are slowly destroyed by the inflammatory process. If left untreated, the teeth lose their ligamentous support to their alveolar bone, become mobile and are eventually lost (Loos *et al.*, 2005).

Traditionally, mechanical/surgical interventions have been the foundation of periodontal therapy. Antibiotics have been used in individuals with advanced periodontal disease who failed to respond adequately to mechanical therapy. It has been established that scaling and root planing (SRP) are anti-infective procedures, as they can reduce bacteria supra- and subgingivally (Anwar *et al.*, 1992; Bodinka *et al.*, 1994; Pradeep and Kathariya, 2011), but major pathogens may escape the antibacterial effect of SRP by invading periodontal tissues, residing in concavities not reached by instruments, or because of poor host resistance (Rabbani *et al.*, 1981). Therefore, the objectives of systemic antibiotic therapy are to reinforce mechanical periodontal treatment for bacterial elimination and to support the host defense system by killing subgingival pathogens not affected by SRP. Several antimicrobial agents [e.g., clarithromycin (Pradeep and Kathariya, 2011), metronidazole (MTZ; Lindhe *et al.*, 1983), amoxicillin plus MTZ (Cionca, 2009)] have been tested for systemic use in periodontal therapy.

Metronidazole (Lindhe *et al.*, 1983; Cionca, 2009; Loesche *et al.*, 1991; Van Oosten *et al.*, 1987) and related nitroimidazole derivatives, including ornidazole (Pradeep *et al.*, 2012; Mombelli *et al.*, 1989) and

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tinidazole (Pritchard *et al.*, 1987), have a spectrum of activity against strictly anaerobic microorganisms and have been used successfully in the treatment of periodontal diseases. Satranidazole (SZ) is another antibiotic that belongs to the 5-nitroimidazole group. Satranidazole [1-methylsulphonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone] is a novel nitroimidazole that differs from other 5-nitroimidazoles such as MTZ, ornidazole, and tinidazole in that the 2C of the imidazole ring is connected via nitrogen to a substituted imidazolidinone moiety (Nair *et al.*, 1983). It possesses superior activity to MTZ against caecal amebiasis in the mouse model (Ray *et al.*, 1982). The MIC90 of SZ was found to be fourfold lower than MTZ against 50 clinical isolates of anaerobes (Gowrishankar *et al.*, 1985). Satranidazole has been shown to damage DNA as a consequence of reduction of the nitro group (Zahoor *et al.*, 1986). Pharmacokinetic studies of SZ in humans have demonstrated a longer half-life (SZ, 14 hours; MTZ, 8 hours) and higher blood levels than MTZ. This necessitates less frequent dosing of SZ as compared to MTZ.

Periodontal pathogens such as *Porphyromonas gingivalis, Tannerella forsythia and Aggregatibacter actinomycetemcomitans* are more effectively reduced by the use of systemic antibiotics and SRP than by SRP alone (Loesche *et al.*, 1992; Winkel *et al.*, 1997; Carvalho *et al.*, 2005). Satranidazole has been reported to be more active towards anaerobes than many 5-nitroimidazoles because of its relatively high redox potential, which makes it more resistant to inactivation by oxygen (Zahoor *et al.*, 1986). These factors combined with its greater potency are believed to contribute to reducing the mean counts of periodontopathogens. Among the factors related to systemic antimicrobial usage in the treatment of periodontal diseases, adverse effects should be taken into account; in particular, side effects for individual patients, as well as the increase in bacterial resistance, which is a major global public health problem. These factors should be considered when prescribing systemic antimicrobials and they should not be used routinely, but rather in certain patients and under defined periodontal conditions (Herrera *et al.*, 2002; Lindhe *et al.*, 2002).

To the best of our knowledge, no studies to date have evaluated the efficacy of systemic SZ in CP. Hence, the present study was designed to determine whether the use of adjunctive systemic SZ improves the clinical and microbiological outcomes in CP subjects.

Materials and methods

Source of data

The subjects for this study were selected from the outpatient section of the Department of Periodontics, Government Dental College and Research Institute, from June to November of 2011. Sixty-six subjects, aged 30 to 50 years (34 males and 32 females) and diagnosed with chronic periodontitis, were enrolled in this study. It was made clear to the potential subjects that participation was voluntary. They were thoroughly informed of the nature, potential risks and benefits of their participation in the study. They were also informed about the known side effects of SZ in relation to alcohol and possible gastrointestinal disturbances. Written informed consent was obtained from patients, and ethical clearance for the study was received from the Institutional Ethical Committee and Review Board, Government Dental College and Research Institute.

Inclusion and exclusion criteria

Sixty-six systemically healthy subjects with untreated moderate to advanced periodontitis were recruited into the study based on the following criteria: age 30 to 50 years, the presence of at least 12 scorable teeth (not including third molars and teeth with orthodontic appliances, bridges, crowns, or implants) with a probing depth (PD) $\geqslant$ 5 mm and/or CAL $\geqslant$ 4 mm and radiographic evidence of bone loss.

Exclusion criteria included pregnancy or lactation, smokers, systemic diseases (e.g., diabetes mellitus), immunocompromised patients, subjects who had taken systemic antibiotics within the previous 6 months, use of non-steroidal anti-inflammatory drugs, confirmed or suspected intolerance to 5-nitroimidazole derivatives, and sub-gingival SRP or surgical periodontal therapy in the previous year.

The clinical parameters to be recorded included: plaque index (PI; Löe, 1967), gingival index (GI; Glavind and Löe, 1967), clinical attachment level (CAL) measured to the nearest mm from the cemento-enamel junction (CEJ) to the deepest probeable point using a standardized periodontal probe (UNC 15 periodontal probe, Hu-Friedy, IL, USA) and PD taken from the gingival margin to the bottom of the pocket.

Treatment protocol

The subjects were randomly allocated to one of the two groups using a computer-generated table of random numbers to receive one of the two treatments. The computerized generation of random allocation sequence and the allocation of subjects were done by the chief investigator (ARP). Group 1 consisted of 33 subjects and Group 2 had 33. Supra-gingival scaling was performed in all subjects one week before the baseline visit. Strict oral hygiene instructions were given to all subjects at the same time by the operator/clinical examiner (PN). Clinical examiner calibration was performed on 20 patients and the intra-examiner agreement was 95.2 %.

At the baseline visit, the clinical parameters mentioned earlier were recorded in four teeth with the most severe destruction both clinically and radiographically at 6 sites/tooth (mesio-buccal, mid-

Table 1. Probing depth (PD) and clinical attachment loss (CAL) for Groups 1 and 2 (mean ± SD) at different time intervals

Parameter	Visits	Group 1	Group 2
PD (mm)	Baseline	7.71 ± 0.98	7.91 ± 0.92
	1 month	6.75 ± 1.08	5.52 ± 1.07
	3 months	6.13 ± 1.06	4.51 ± 1.09
	6 months	6.32 ± 0.98	4.17 ± 1.12
CAL (mm)	Baseline	8.62 ± 0.85	8.42 ± 0.72
	1 month	8.12 ± 0.95	6.21 ± 1.15
	3 months	7.85 ± 1.29	5.67 ± 1.37
	6 months	7.51 ± 1.30	5.22 ± 1.42

Group 1, scaling and root planing + placebo; Group 2, scaling and root planing + satranidazole

buccal, disto-buccal, mesio-palatal/lingual, mid-palatal/lingual and disto-palatal/lingual) in each subject by the same person (PN). Next, the operator treated the periodontally diseased sites with thorough SRP to the depth of the pocket under local anaesthesia. Afterwards, each subject received a package containing SZ or placebo medication; all packages were identical in appearance and were marked only with the subject number. Subjects in Group 2 received SZ (Satrogyl tablets, Alkem Laboratories Ltd., Mumbai, India) 300 mg to be taken twice daily for 10 days, while subjects in Group1 received placebos similar in appearance and the after containing same constituents as SZ except the active ingredient and given a similar regimen. A standard form was given to each subject to record any side effects. The patient's compliance with the prescribed regimen was checked by asking the patient to bring the used tablet packet to the next visit. The treatment group was concealed from the patient, clinical examiner, operator, and statistician.

Clinical monitoring

All the subjects in both groups were recalled at 1 month, 3 months and 6 months after the baseline visit. Clinical parameters, including PI, GI, PD and CAL were recorded at these time intervals. Oral hygiene instructions were checked and reinforced at each visit. The standard form was also checked at every interval to note any side effects.

Microbiological analysis

The microbial analysis was done at baseline, 3 and 6 months using polymerase chain reaction (PCR) using DNA polymerase.

Plaque sample collection

Subgingival plaque was removed with a sterile curette (Gracey Curettes, Hu-Friedy, Chicago, IL, USA), starting at the most apical extent of the deepest periodontal pocket. The samples were not pooled and only the plaque sample from the single deepest pocket was analyzed. Collected samples were placed in airtight plastic vials with 500 µl of Tris EDTA (TE) buffer and were immediately transferred to the laboratory for microbiological analysis using PCR technology.

PCR detection

The samples were stored at -20º C to be processed immediately. Samples collected at baseline, 3 and 6 months were analyzed by PCR. Species-specific PCR assays were performed to detect *T. forsythia, P. gingivalis*, and *A. actinomycetemcomitans.*

For *T. forsythia* (Slots *et al.*, 1995), denaturation at 95º C for 2 minutes was followed by 36 cycles of denaturation at 95º C for 30 seconds, annealing at 60º C for 1 minute, extension at 72º C for 1 minute, and a final elongation step at 72º C for 2 minutes. For *P. gingivalis* (Benkirane *et al.*, 1995), denaturation at 94º C for 5 minutes was followed by 30 cycles of denaturation at 94º C for 1 minute, annealing at 70º C for 1 minute, extension at 72º C for 1 minute, and a final elongation step at 72º C for 2 minutes. For *A. actinomycetemcomitans* (Ashimoto *et al.*, 1996) denaturation at 95º C for 2 minutes was followed by 36 cycles of denaturation at 94º C for 30 seconds, annealing at 55º C for 1 minute, extension at 72º C for 2 minutes, and a final elongation step at 72º C for 10 minutes.

Primers

Species-specific PCR assays were performed to detect *T. forsythia, P. gingivalis* and *A. actinomycetemcomitans.* Primers used for *T. forsythia* (Slots J *et al.*, 1995): 5' GCG TAT GTA ACC TGC CCG CA 3', 5' TGC TTC AGT GTC AGT TAT ACC T 3'; *P. gingivalis* (Benkirane *et al.*, 1995): 5' AAT CGT AAC GGG CGA CAC AC 3', 5' GGG TTG CTC CTT CAT CAC AC 3'; and *A. actinomycetemcomitans* (Ashimoto *et al.*, 1996): 5' AAA CCC ATC TCT GAG TTC TTC TTC 3', 5' ATG CCA

Table 2. Decrease in probing depth (PD) and clinical attachment loss (CAL) gain (mean ± SD) between baseline and different time intervals for Groups 1 and 2

Parameter	Visits	Group 1	Group 2	*p* value
PD (mm)	1 month	0.96 ± 1.19	2.41 ± 1.09	0.001*
	3 months	1.63 ± 1.55	3.55 ± 1.54	0.001*
	6 months	1.42 ± 1.01	3.84 ± 1.31	0.001*
CAL (mm)	1 month	0.51 ± 1.25	2.24 ± 1.21	0.001*
	3 months	0.83 ± 1.25	2.87 ± 1.66	0.001*
	6 months	1.15 ± 1.49	3.22 ± 1.01	0.001*

*Statistically significant at 5% level of significance ($p < 0.05$). Group 1, scaling and root planing + placebo; Group 2, scaling and root planing + satranidazole

Table 3. Number of patients positive by PCR assay for each species

	Time	Group1	Group 2	*p* value
P. gingivalis	Baseline	28	30	NS
	3 months	22	18	0.001*
	6 months	25	11	0.001*
T. forsythia	Baseline	26	28	NS
	3 months	20	18	0.001*
	6 months	23	10	0.001*
A. actinomycetemcomitans	Baseline	22	25	NS
	3 months	17	14	0.001*
	6 months	20	06	0.001*

*Statistically significant at 5% level of significance ($p < 0.05$). NS, not significant.

ACT TGA CGT TAA AT 3'.

Primary and secondary outcome measures

The primary outcome of the study was gain in CAL. The differences in PI, GI, PD and reduction in the number of patients harboring periodontopathogens between the two groups were taken as the secondary outcomes.

Statistical analysis

Power analysis calculations were performed before the study was initiated. To achieve 90% power and detect mean differences between groups, 30 subjects in each group were required. Continuous variable (PI, GI, PD, CAL and microbiological count) were expressed as mean ± standard deviation (SD). Normality assumption was tested using Shapiro-Wilk's W test. Between treatment group comparison were carried out using the Mann-Whitney test. Wilcoxon signed ranks test was used for comparison within the SZ and control groups, respectively. Statistical significance was defined as $p < 0.05$. Statistical analysis was performed with statistical software SPSS version 10.5 (SPSS, Chicago, IL).

Results

Figure 1 shows a CONSORT flowchart exhibiting the number of subjects enrolled, excluded and analyzed. Three subjects from Group 1 and three from Group 2 failed to follow up to the end of the study. In all 60 subjects (33 males, 27 females) were finally analyzed.

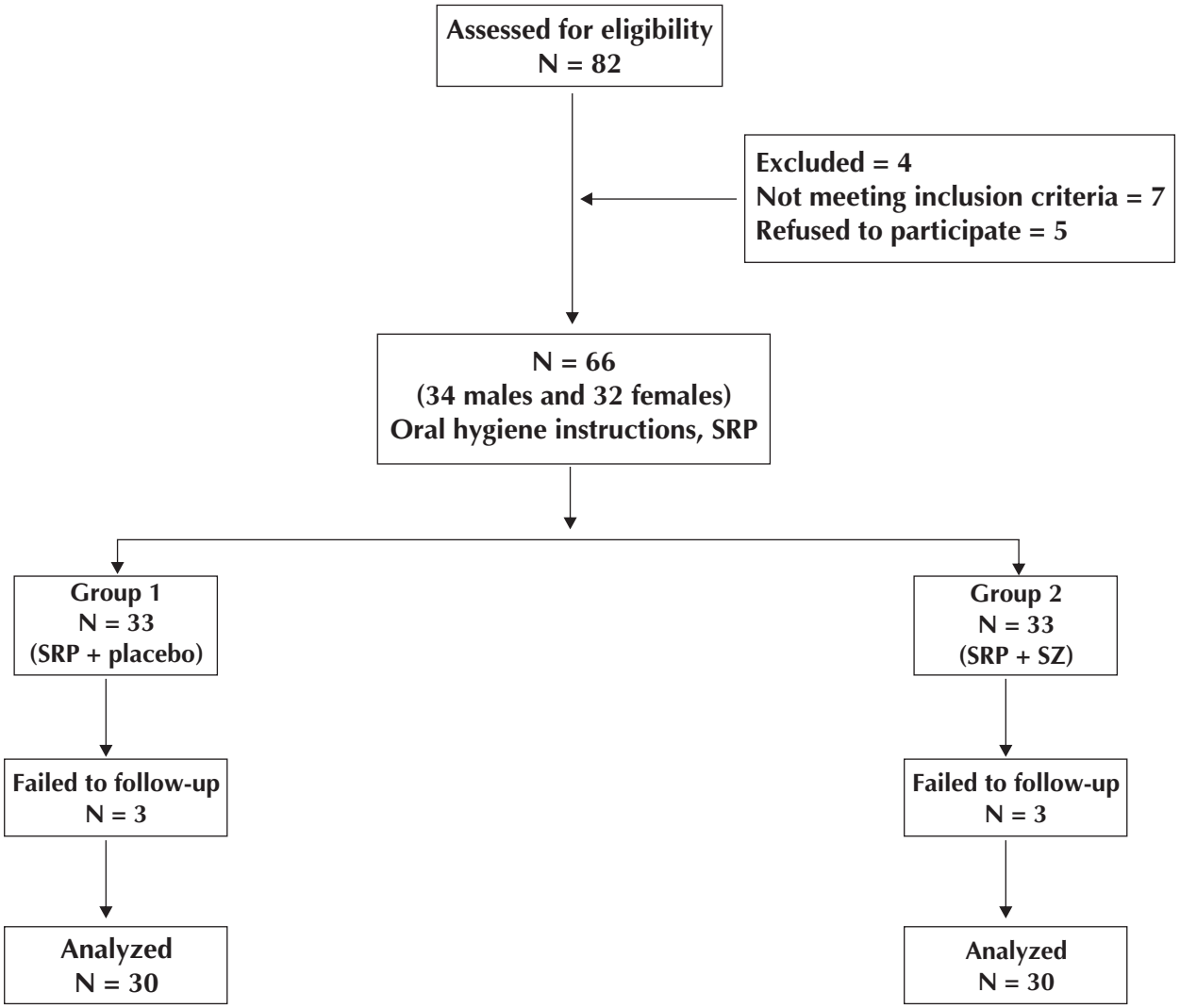
Plaque index and gingival index

There was reduction but no significant difference was found between the two groups in PI and GI at any point.

Probing depth

The decrease in PD was statistically significant within both groups compared to baseline at all time intervals *(Tables 1 and 2)*. When the groups were compared to each other, the decrease in PD at each time period was

Figure 1. Study flow chart. SRP, scaling and root planing; SZ, satranidazole.



statistically significant.

Clinical attachment loss

The difference from baseline was statistically significant in both groups. Clinical attachment gain was greater in Group 2 compared to Group 1 at all time points, and the difference reached the level of statistical significance *(Tables 1 and 2)*.

Microbial analysis

The PCR analysis showed a significant difference in the number of patients harboring periodontopathogens at 3 and 6 months ($p < 0.05$). At baseline the inter-group difference was not significant ($p > 0.05$). At 6 months there was a slight increase in the number of patients harboring periodontopathogens in Group 1. A significant reduction ($p < 0.05$) in the number of patients harboring periodontopathogens (*P. gingivalis, T. forsythia, A. actinomycetemcomitans*) was found in Group 2 compared to Group 1 at all time points except baseline (*Table 3*).

Adverse drug reactions

Three subjects (2 females, 1 male) in Group 2 reported slight taste alteration. A female patient in Group 2 complained of nausea during the 10-day regimen.

Discussion

The findings of the present clinical trial demonstrated that in patients with advanced periodontal disease systemic administration of SZ resulted in an improvement of the periodontal conditions. The combined mechanical and systemic antibiotic therapy (Group 2) was more effective than mechanical therapy alone (Group 1) in terms of improvement of clinical and microbiological features of periodontal disease.

Systemic medications of value in periodontal therapy can be divided into two major categories: those that are directed against periodontal pathogens and those that modulate the host response. Antibiotics and other chemotherapeutic agents are usually prescribed for patients who do not respond to conventional

mechanical therapy, or as an adjunct to periodontal surgery. A number of periodontal benefits have been associated with systemic medications as an adjunct to SRP, including reductions in PD, gain in CAL, long-term reduction of periodontal pathogens, elimination of invasive pathogens in periodontal tissues and a decrease in the extent and severity of periodontal surgery (Ciancio *et al.,* 2002). The finding that SRP combined with administration of systemic SZ was more effective than mechanical therapy alone in terms of eliminating deep pockets (⩾ 6 mm) and promoting CAL gain at such sites is in agreement with results reported in previous studies (Pradeep and Kathariya, 2011; Pradeep *et al.*, 2012; Carvalho *et al.,* 2004).

Metronidazole (Lindhe *et al.*, 1983) and related nitroimidazole derivatives, including ornidazole (Pradeep *et al.*, 2012) and tinidazole (Pritchard *et al.*, 1987), have been successfully used in the treatment of CP. Local drug delivery of SZ resulted in reduction of GI, PD and gain in CAL in the treatment of CP. Also, the clinical effectiveness of SZ showed better results in comparison with MTZ (Bansal *et al.*, 2004). There has been no study to test the efficacy of systemic SZ, which belongs to the nitroimidazole group, in the treatment of CP. Hence SZ was selected as an adjunct to SRP to test its systemic efficacy in the treatment of CP.

Scaling and root planing is effective for the majority of patients with mild to moderate CP. In a thorough evidence-based review, Cobb (1996) calculated the mean PD reduction and gain in CAL that can be achieved with SRP at sites that initially were 4 to 6 mm in depth and 7 mm or greater in depth. He reported mean PD reductions of 1.29 mm and 2.16 mm, respectively, and mean gain in CAL of 0.55 mm and 1.29 mm, respectively. However, a study by Sbordone *et al.*, (1990) showed that the improvement in clinical parameters achieved with SRP could not last long, and there was a return to baseline values within 2 months. Caffesse *et al.*, (1996) demonstrated that when pockets exceeded 5 mm, clinicians often failed to adequately debride root surfaces and removed deposits completely only 32 percent of the time.

In our study, use of SZ as an adjunct to SRP resulted in a statistically significant clinical improvement over SRP alone. For sites with an initial PD of 7 mm or greater, the mean CAL gain was 3.22 mm following SRP plus SZ and 1.15 mm following SRP alone, for a difference of 2.07 mm. The mean amount of PD reduction was 3.84 mm following SRP plus SZ and 1.42 mm following SRP alone, for a difference of 2.42 mm. These findings suggest that the combined effect of SZ and SRP achieved statistically significant results with regard to PD reduction and CAL gain.

In a 3-month trial Carvalho *et al.* (2004) concluded that clinical outcomes of non-surgical periodontal therapy improved significantly when systemic MTZ (400 mg tid x 10 days) was administered to CP subjects. The improvements in clinical parameters in our study are in accordance with this study. Thus, there is a possibility of equal efficacy of both treatments. However, less frequent dosing of SZ is required as compared to MTZ.

The results of systemic MTZ and amoxicillin (mean PD = 4.4 mm) significantly improved the 6-month clinical outcomes of full-mouth non-surgical periodontal debridement in CP subjects (Cionca, 2009). The improvement in clinical parameters in our study was much greater because PD reduction usually is greater at sites with larger initial PD (Becker *et al.,* 1998; Pihlstrom *et al.*, 1981). The results of the present study are also in accordance with one of our recent trials of systemic ornidazole in CP (Pradeep *et al.*, 2012).

The effect of SRP combined with SZ led to a significant reduction in the number of patients harboring periodontopathogens *P. gingivalis, T. forsythia* and *A. actinomycetemcomitans* at all time periods, compared to baseline. Hence, SZ was found to be effective against the tested anaerobes. Scaling and root planing alone had a limited effect on these periodontopathogens, and there was an increase in the number of patients with single sites harboring these periodontopathogens at 6 months when compared with the 3-month results in Group 1. The finding of the present investigation that systemically administered SZ decreased the number of patients with a single site harboring the three periodontopathogens, and that SRP alone is ineffective in eliminating the pathogens is in accord with the findings of other studies (Loesche *et al.,* 1992; Winkel *et al.*, 1997; Carvalho *et al.*, 2005). Hence, the microbiological data suggest a benefit to the combined use of SZ and SRP in reducing the number of patients with single sites harboring the three periodontopathogens contributing to the etiopathogenesis of periodontal diseases.

An advantage of SZ is that it has a significantly lower incidence of side effects, making it very convenient for the patient to take the drug (Ciancio *et al.*, 2002). Hence, SZ may be more likely to have a better rate of patient compliance, resulting in increased effectiveness of drug therapy for CP. However, it will be important to examine long-term clinical changes and to investigate other microorganisms that have been postulated to contribute to the etiopathogenesis of periodontal diseases in larger groups of subjects to substantiate the clinical usefulness of combined periodontal therapies.

In conclusion, this study has shown that the systemic use of SZ, when used in conjunction with initial periodontal treatment consisting of SRP in adult subjects with periodontitis, achieves significantly better clinical and microbiological results than initial periodontal treatment alone. Further long-term, multi-center longitudinal trials are required to assess and establish the efficacy of systemic SZ in the management of CP, and to compare this treatment protocol with other established drugs of this group.

Acknowledgments

The authors express their gratitude to Alkem Laboratories Ltd., Mumbai, India for providing gift samples of Satrogyl 300 mg and placebo tablets. The authors also express their thanks to Mr. Manjunath Sharma, Bangalore, for carrying out the required statistics.

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