Efficacy of Subgingivally Delivered Satranidazole in the Treatment of Type 2 Diabetes Subjects with Chronic Periodontitis: A Randomized Controlled Clinical Trial

Dr. N Priyanka[1], Dr. Nitish Kalra[1], Dr. Shahabe Saquib[2], Dr. Jadhav Varsha[2], Dr. Davangere Umashree[3], Dr. Malgaonkar Nikhil[4], Dr. A R Pradeep[1]

[1]Department of Periodontics, Government Dental College and Research Institute, Bangalore, Karnataka; [2]Departments of Periodontics, [3]Prosthodontics, and [4]Oral Pathology, Yogita Dental College and Hospital, Khed, Ratnagiri, Maharashtra, India

Abstract

Background: The present clinical trial was designed to investigate the effectiveness of subgingivally delivered satranidazole (SZ) gel as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis.

Methods: Sixty-four subjects with probing depth (PD) ≥ 5 mm and who were diagnosed with type 2 diabetes were selected. Thirty-two subjects each were randomly assigned to SRP + placebo (Group 1) and 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.

Results: At 6 months, Group 2 had greater mean reduction (4.73 mm) in PD as compared to Group 1 (2.09 mm; $p < 0.05$) and also a greater mean CAL gain (3.92 mm versus 1.64 mm; $p < 0.05$).

Conclusion: The use of 3% SZ gel, when used as an adjunct to non-surgical periodontal therapy in subjects with periodontitis, achieves significantly better clinical results than initial periodontal treatment alone.

Key words: Satranidazole, chronic periodontitis, local drug delivery, diabetes

Introduction

Periodontal diseases are chronic infectious diseases characterized by a bacterial challenge that can provoke a destructive host response leading to periodontal attachment loss and ultimately possible tooth loss (Kornman and Loe, 1993).

Diabetes mellitus (DM) is a chronic disease characterized by dysregulation of carbohydrate, protein, and lipid metabolism. An elevation of blood glucose level (hyperglycemia) is the primary feature of DM and results from a defect in insulin secretion by pancreatic beta cells, a decrease in insulin sensitivity, or a combination of both. The most common form of DM is type 2 DM, which accounts for 85% of all diabetes patients (Mealy *et al.*, 2003).

Periodontitis is more prevalent and severe among patients with DM2 than among healthy controls (Campus *et al.*, 2005; Jansson *et al.*, 2006; Taylor and Borgnakke, 2008). Thus, DM2 may initiate or deteriorate periodontitis. However, the reverse could also be true: periodontitis may initiate or deteriorate DM2. The strongest support for this comes from studies showing that treatment of periodontitis improves glycemic control in DM2 patients (Grossi, 2001; Taylor, 2003; Kiran *et al.*, 2005)

Steinberg *et al.* (1990) reported that the repeated, long-term use of systemic antibiotics was fraught with potential dangers, including resistant strains and superimposed infections and problems such as lack of patient compliance. Conversely, Jorgensen and Slots (2000), showed that local drug delivery can provide 100-fold higher therapeutic doses of the agent in subgingival areas than systemic therapy. Therefore, the local administration of antimicrobials provides a useful solution to the complications of systemic antibiotics.

Correspondence to: Dr. Priyanka N MDS, Department of Periodontics, Government Dental College and Research Institute, Bangalore-560002, Karnataka, INDIA Email: priyanka.n7@gmail.com

Several antimicrobial agents [e.g., tetracycline, metronidazole (MTZ; Kinane and Radvar, 1999), clarithromycin (Agarwal *et al.*, 2012), azithromycin (Pradeep *et al.*, 2008)] have been tested for local drug delivery use in periodontal therapy. Metronidazole and related nitroimidazole derivatives, including ornidazole (Pradeep *et al.*, 2012) and tinidazole (Pritchard and Higgins, 1987), have a spectrum 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 which differs from other 5-nitroimidazoles such as MTZ, ornidazole, and tinidazole, in that the 2C of the imidazole ring is connected via a nitrogen to a substituted imidazolidinone moiety (Nair and Nagarajan, 1983). 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. These factors combined with its greater potency are believed to contribute to its therapeutic efficacy (Nair and Nagarajan, 1983).

In a similar study, Bansal *et al.* (2009), concluded there were improved clinical outcomes with local drug delivery of SZ in chronic periodontitis. We hypothesized similar benefits with local drug delivery of SZ in the treatment of DM2 patients with chronic periodontitis. To the best of our knowledge, there is no published literature on evaluation of clinical efficacy of *in situ* gel using SZ in DM2 patients with chronic periodontitis. Keeping the above facts in mind, the aim of this double-blinded, placebo-controlled randomized clinical trial was to evaluate the clinical efficacy of subgingivally delivered SZ in DM2 patients with chronic periodontitis.

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, Bangalore, from March 2012 to September 2012. Sixty-four patients, aged 30 to 50 years (36 males and 28 females) and who were diagnosed with DM2 and chronic periodontitis were enrolled in this study. It was made clear to the potential subjects that participation was voluntary. Written informed consent was obtained from subjects, and ethical clearance for the study was received from the Institutional Ethical Committee and Review Board, Government Dental College and Research Institute.

Selection criteria

Well-controlled DM2 patients (Hb A_{1c} < 8%) were selected and classified based on criteria given by the American Diabetic Association in 2011 and glycated hemoglobin levels. Type 2 diabetic subjects with PD ≥ 5 mm and/or CAL ≥ 4 mm and vertical bone loss ≥ 3 mm on intraoral periapical radiographs and no history of antibiotic or periodontal therapy in the preceding 6 months were included. Patients with known or suspected allergy to the SZ group, those on systemic antimicrobial therapy, patients with aggressive periodontitis, smokers, alcoholics, immunocompromised patients, and pregnant or lactating females were excluded.

Sixty-nine subjects were initially analyzed for the study. Five subjects were excluded because they did not meet the inclusion criteria. After subject selection (by ARP), 32 subjects were randomly (by computer generated system) assigned to each treatment group, and one site per subject was treated with SRP plus placebo (Group 1) or SRP plus SZ (3%/0.1 ml) *in situ* gel (Group 2). Scaling and root planing was performed at baseline until the root surface was considered smooth and clean by the operator (PN). No antibiotics or antiplaque and anti-inflammatory agents were prescribed after treatment.

Clinical parameters, including gingival index (GI; Glavind and Löe, 1967), plaque index (PI; Löe, 1967), PD, and CAL were recorded at baseline (before SRP) and at 3 and 6 months. A custom-made acrylic stent and a University of North Carolina no. 15 color-coded periodontal probe (UNC 15 periodontal probe, Hu-Friedy, IL, USA) were used to standardize the measurement of PD and CAL. Clinical attachment loss was calculated by measuring the distance from the stent (apical extent) to the base of the pocket minus the distance from the stent to the cemento-enamel junction.

A single clinician (PN) provided treatment to both groups, and all pre- and post-treatment clinical parameters were recorded by another examiner (ARP) who was masked to the type of treatment received by the subjects.

Intra-examiner calibration

Intra-examiner calibration was achieved by examination of 20 patients twice, 24 h apart, before beginning the study. Calibration was accepted if measurements at baseline and 24 h were similar to 1 mm at the 95% level.

Primary and secondary outcome measures:

The primary outcome of the study was CAL. The secondary outcomes included PI, GI and PD.

Formulation of 3% SZ in situ gel

After intensive *in vitro* investigations for optimization and stability at the collaborative center (Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bangalore, India), the formulation described below was developed.

The SZ gel (3%) was prepared as described in a previous study (Bansal *et al*., 2009). Weighed carbopol 934P was dissolved in 50 mL of McIlvaine buffer, pH 6.6. The SZ drug was also dissolved in about 25 mL of McIlvaine buffer. This solution of SZ was slowly added to a solution of CB 934P with stirring. Then, the gelling agent sodium carboxy methylcellulose (SCMC) was added slowly with continuous magnetic stirring at 100 rpm. The volume was made up to 100 mL with McIlvaine buffer, pH 6.6. The prepared gel was kept for 24 h at room temperature for complete polymer dissolution.

Local drug delivery

For standardization, 0.1 mL prepared SZ gel (3%) was injected into the periodontal pockets using a syringe with a blunt cannula. No periodontal dressing was applied after delivery of the drug because the prepared formulation decreases in viscosity, which causes swelling and occlusion of the periodontal pocket.

After placement of the *in situ* gel, subjects were instructed to refrain from chewing hard or sticky foods, brushing near the treated areas, or using any interdental aids for 1 week. Adverse effects were noted at recall visits, and any supragingival deposits were removed.

Collection of gingival crevicular fluid (GCF) samples

Gingival crevicular fluid was collected from drug-delivery sites in six randomly selected patients from Group 2 (SRP + SZ) using 5 μL volumetric microcapillary pipettes calibrated in 1 μL increments (Sigma-Aldrich, St. Louis, MO) at baseline; at 2, 4, 6, 24 and 48 hours; and at weeks 1, 2, 3, and 4. Collected samples were stored at 4^0 C to 8^0 C until the estimation was done.

Estimation of quantity of SZ

The drug quantity estimation was done using gradient reverse phase high-performance liquid chromatography (HPLC; 1200 Series, Agilent Technologies, Palo Alto, CA) with pumps, a variable wavelength programmable ultraviolet/visible spectroscopy detector and a system controller. An operating software data station (Agilent Dissolution Testing UV-visible ChemStation Software (G1118AA), Agilent Technologies) was used.

Chromatographic conditions

A column (150 mm [length], 4.6 mm [internal diameter] and particle size of 5 mm; Agilent HPLC columns-Zorbax Column Compartment SL support, Agilent Technologies) was used as the stationary phase. The mobile phase consisted of 35% volume of buffer (0.1% phosphoric acid) and 65% volume of acetonitrile (volume/volume). The mobile phase was filtered through a 0.45 mm membrane filter (Sartorius, Goettingen, Germany) and sonicated to remove air bubbles. The flow rate was 1.0 mL/minute, and the column effluent was monitored at 238 nm.

Calibration curve in GCF

A standard stock solution of SZ (1 mg/mL) was prepared in acetonitrile in a 100 mL volumetric flask, adding 30 to 40 mL diluent prepared by mixing 40% by volume of 1.4 g/L solution of dihydrogen phosphate, pH 4, with phosphoric acid and 60% by volume of acetonitrile. The flask was sonicated to dissolve the solvents. The standard stock solutions were diluted 100 times to get a concentration of 10.4 mg/mL by using the GCF stock solution. GCF stock solution was prepared by spiking the GCF samples from 10 capillary tubes, obtained at baseline from patients in Groups 1 and 2 for standardization, to the 1 mL solution that contained acetonitrile. An aliquot of 80 μL working stock solution (10.4 mg/mL) was added to 20 μL 1% phosphoric acid buffer (in the pH range of 4) in microcentrifuge tubes and vortexed for 1 minute. Acetonitrile was used as an extracting solvent, and 1 mL was taken for the extraction of SZ. The microcentrifuge tubes were vortexed for 2 minutes and then centrifuged at 10,000 revolutions per minute (rpm) in a cold centrifuge for 10 minutes. After centrifugation, an aliquot of 20 μL supernatant solution was injected via HPLC. The amount of SZ present in a capillary tube was determined by comparing the peak responses of the standard and the sample of SZ solution.

Sample preparation

Gingival crevicular fluid was transferred to a 5 μL centrifuge tube containing 80 μL acetonitrile. Eighty μL of GCF (after transfer) and 20 μL buffer (1% phosphoric acid) were combined in a microcentrifuge tube and vortexed for 1 minute. One mL of acetonitrile was added to the above mixture and it was vortexed for 1 minute. Then the solution was centrifuged at 10,000 rpm in a cold centrifuge for 10 minutes. After centrifugation, an aliquot of 20 μL supernatant solution was injected via HPLC, and the chromatogram was recorded. The amount of SZ present in the GCF was determined by comparing the peak responses of the standard and the sample of SZ solution.

Statistical analysis

Power analysis calculations were performed before the study was initiated. To achieve 90% power and detect mean differences of the clinical parameters between groups, 30 sites in each group were required. Continuous variables (PI, GI, PD, CAL) were expressed as mean ± standard deviation (SD). Normality assumption was tested using Shapiro-Wilk's W test. Between the treatment groups comparison was carried out using 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

A CONSORT flowchart exhibiting the number of subjects finally analysed and those dropping out is shown in *Figure 1*. Fifty-seven of 64 subjects completed the study. Seven subjects did not follow up after the baseline examination. Fifty-seven treatment sites (one site/subject) were evaluated for clinical parameters at baseline, 3 and 6 months. *Table 1* gives the descriptive statistics (age, number and sex) of the population.

Table 1. Descriptive statistics of the population.

PARAMETER	Group 1	Group 2	*p* value
Age (years, mean ± SD)	42.2 ± 8.2	40.3 ± 10.2	NS
Number of participants	29	28	NS
Males	16	14	NS
Females	13	14	NS

Clinical evaluation

No adverse reaction was observed in any subject from the test group, and no patient reported any discomfort. Healing was uneventful. All subjects tolerated the drug, without any post-application complications.

There was reduction but no significant difference was found between the two groups in PI and GI at any point. However, the decrease in GI was statistically significant within both groups at 3 months (*Table 2*). The decrease in PD was statistically significant within both groups compared to baseline at all time intervals (*Tables 3* and *4*). When the groups were compared to each other, the decrease in PD at each time period was statistically significant. The difference in CAL from baseline was statistically significant in both groups; CAL gain was greater in Group 2 compared to Group 1 at all periods, and the difference reached the level of significance (*Tables 3* and *4*).

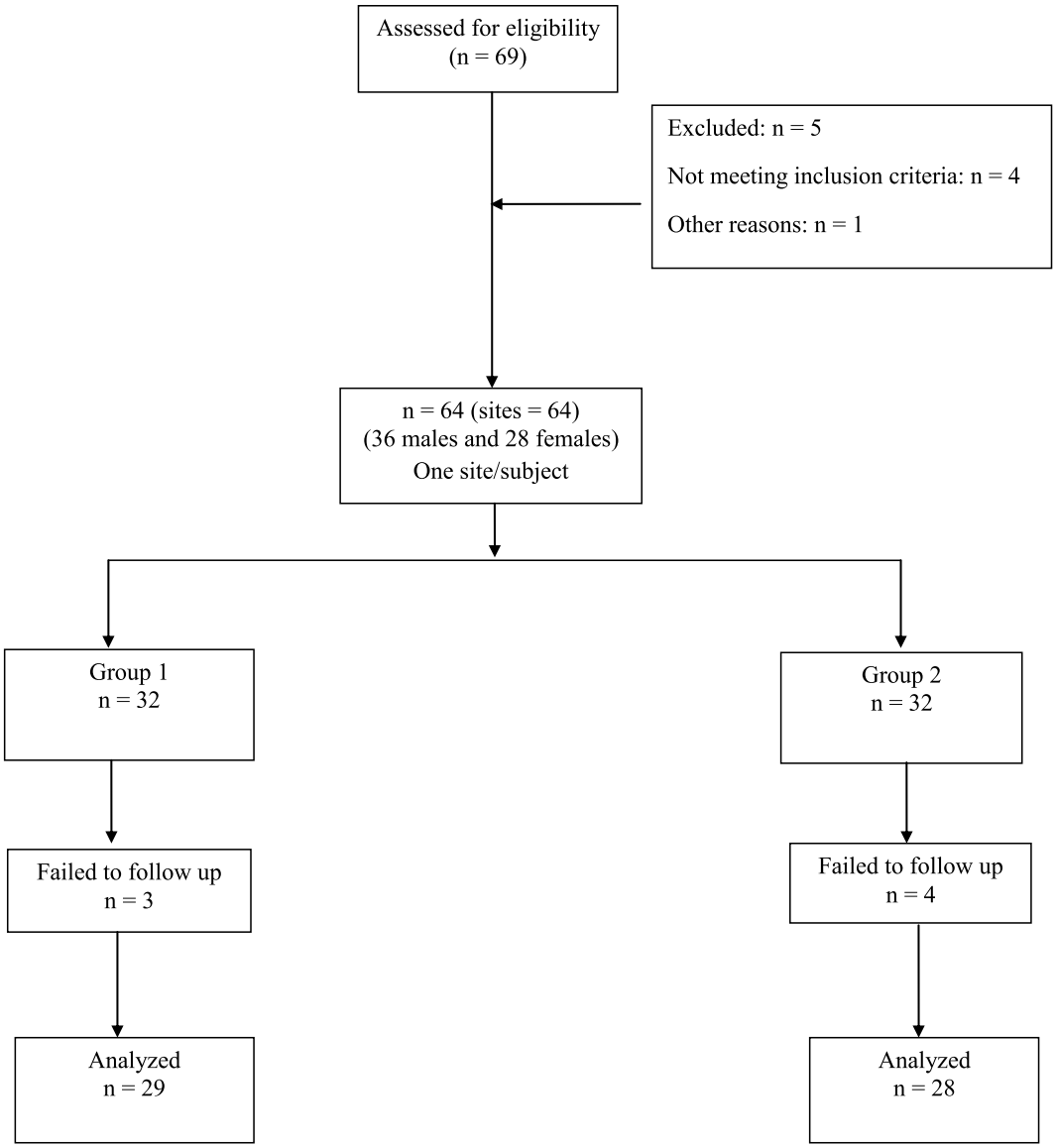

Figure 1: Study flow chart. Group 1 received scaling and root planing (SRP) and local delivery of a placebo gel. Group 2 received SRP + 3% satranidazole gel.

Table 2. Mean ± SD and *p* values for plaque index (PI) and gingival index (GI) in the two groups at various intervals. Group 1 received scaling and root planing (SRP) and local delivery of a placebo gel. Group 2 received SRP + 3% satranidazole gel.

Parameter	Visits	Group 1	Group 2	*p* value
Plaque index	Baseline	2.98 ± 0.22	2.86 ± 0.17	NS
	1 month	2.67 ± 0.16	2.53 ± 0.15	NS
	3 months	2.62 ± 0.14	2.56 ± 0.22	NS
	6 months	2.71 ± 0.25	2.68 ± 0.16	NS
Gingival index	Baseline	2.66 ± 0.25	2.65 ± 0.23	NS
	1 month	2.36 ± 0.27	2.30 ± 0.27	NS
	3 months	1.97 ± 0.23	1.61 ± 0.22	0.001*
	6 months	1.86 ± 0.17	1.44 ± 0.27	NS

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

Table 3. Probing depth (PD) and clinical attachment loss (CAL) for Groups 1 and 2 (mean ± SD) at different time intervals. Group 1 received scaling and root planing (SRP) and local delivery of a placebo gel. Group 2 received SRP + 3% satranidazole gel.

Parameter	Visits	Group 1	Group 2
PD (mm)	Baseline	8.24 ± 1.32	8.31 ± 1.42
	1 month	7.32 ± 1.25	6.55 ± 1.32
	3 months	6.55 ± 1.22	5.32 ± 1.25
	6 months	6.15 ± 1.11	3.58 ± 1.22
CAL (mm)	Baseline	7.63 ± 1.29	7.92 ± 1.21
	1 month	6.54 ± 1.26	6.13 ± 1.16
	3 months	6.11 ± 1.22	5.42 ± 1.13
	6 months	5.99 ± 1.15	4.00 ± 1.11

Table 4. Decrease in probing depth (PD) and clinical attachment loss (CAL) gain from baseline (mean ± SD) at different time intervals for Groups 1 and 2

Parameter	Visits	Group 1	Group 2	*p* value
PD	1 month	0.92 ± 0.07	1.76 ± 0.01	0.001
	3 months	1.69 ± 0.11	2.99 ± 0.17	0.001
	6 months	2.09 ± 0.21	4.73 ± 0.02	0.001
CAL	1 month	1.09 ± 0.03	1.79 ± 0.05	0.001
	3 months	1.52 ± 0.07	2.50 ± 0.08	0.001
	6 months	1.64 ± 0.14	3.92 ± 0.10	0.001

Table 5. SZ concentration (mean ± SD) in GCF after treatment

Time	Concentration of SZ (µg/ml)
Baseline	0.00 ± 0.00
2 hours	14.67 ± 0.03
4 hours	13.21 ± 0.41
6 hours	12.45 ± 0.11
24 hours	11.62 ± 0.25
48 hours	11.01 ± 0.19
1 week	07.75 ± 0.14
2 weeks	03.13 ± 0.22
3 weeks	01.44 ± 0.19
4 weeks	0.81 ± 0.14

Analysis of SZ concentration in GCF

Satranidazole in GCF peaked at 2 hours after application (14.67 ± 0.03 µg/mL; *Table 5*). The mean concentrations at weeks 1, 2, 3, and 4 indicate that SZ was retained in this target compartment for a long period, suggesting a controlled release of the drug until the 4th week.

Discussion

This study was designed with the aim of assessing the efficacy of local drug delivery of 3% SZ gel as an adjunct to non-surgical periodontal therapy in the treatment of patients with DM2 and chronic periodontitis as compared to a placebo group. To our knowledge, there have been no studies reporting the use of 3% SZ gel as local drug delivery in the treatment of patients with chronic periodontitis with DM2. Therefore, a direct comparison with other studies is not possible.

Conventional therapy of chronic periodontitis is based on the suppression of subgingival infections foci by mechanical debridement, such as scaling and root planing (SRP), or surgical procedures. However, with the recognition that periodontal diseases are associated with specific pathogens, interest has grown in the use of anti-microbial drugs for inhibition of these micro-organisms. Indeed, antibiotics may enhance the effect of mechanical debridement procedures by reducing the recurrence rate of periodontal infection, preventing the systemic extension of infection during the acute phases of periodontitis, and restoring the equilibrium among different bacterial species harbored in the oral cavity (Gordon and Walker, 1993).

Severe periodontitis often coexists with diabetes and is considered to be the sixth complication of the disease (Löe, 1993), as both type 1 and type 2 diabetes patients show a three- to four-fold increased risk of periodontitis (Nelson *et al*., 1990; Emrich *et al*., 1991) Uncontrolled or poorly controlled diabetes is associated with increased susceptibility to oral infections, including periodontitis. The incidence of periodontitis increases with age among diabetic subjects after puberty (Seppala *et al*., 1993; Arrieta-Blanco *et al*., 2003) Periodontal disease may be more frequent and severe in diabetic individuals with more advanced systemic complications (Arrieta-Blanco *et al*., 2003).

In our study, in sites with an initial PD of 7 mm or greater, the mean CAL gain was 3.92 mm following SRP + SZ and 1.64 mm following SRP alone, for a difference of 2.28 mm. The mean amount of PD reduction was 4.73 mm following SRP + SZ and 2.09 mm following SRP alone, for a difference of 2.64 mm. The results of this study indicate that both therapies (SRP + placebo gel and SRP + 3% SZ gel) resulted in significant improvements, but patients in the SRP + SZ group showed enhanced clinical outcome ($p < 0.05$) over a period of 6 months as compared to SRP alone. The results of the current clinical trial using 3% SZ are in accordance with a similar study by Bansal *et al.* (2009), which showed reduced gingival inflammation, decreased PD and increased CAL. Hence this proved that patients with relatively well controlled diabetes (HbA_{1c}) usually respond to therapy in a manner similar to non-diabetic individuals.

With regard to the dose of SZ used, 3%/0.1 mL per site was injected in the present study. In another study by Bansal *et al.* (2009), various gelling agents were used for local drug delivery of SZ in patients with periodontitis. Among them, SCMC gelling agent in a concentration of 3% w/v has been reported to show the desired balance of mechanical properties (mucoadhesiveness, hardness, adhesiveness, compressibility, and cohesiveness). Also, the *in vitro* release of SZ from gel containing SCMC gelling agent showed long-term, controlled release. Hence, in the present study 3% SZ gel with SCMC as a gelling agent appeared to be more suitable for obtaining long-term release of the drug, assuring a constant and prolonged concentration at the application site.

The mean concentration of SZ at all observed periods (from baseline to 30 days), as estimated by reverse-phase HPLC, provided sufficient anti-inflammatory activity and fulfilled the conditions for a controlled-release device. Maintenance of this concentration of the drug locally for a long duration (6 months) may have been responsible for the additional improvement in PD and CAL in Group 2 compared to Group 1.

Considering this fact, it can be proposed that SZ delivered subgingivally as an adjunct to SRP will be better approach for treatment of periodontal pocket in DM2 patients with chronic periodontitis compared to SRP alone.

Conclusion

This clinical trial demonstrates that local delivery of 3% SZ into the periodontal pocket stimulated a significant increase in the PD reduction and CAL gain, compared to placebo gel as an adjunct to SRP in DM2 patients with chronic periodontitis. Further, long-term, randomized, multi-center, double-blinded clinical trials with microbiologic profiles and assessment of improvement in glycemic control are needed to confirm the findings in this study.

Acknowledgments

The authors express their gratitude to Alkem Laboratories Ltd., Mumbai, India for providing a gift sample of SZ. The authors also express their gratitude to Dr. B. G. Shivananda (Principal) and Mr. M. Nagraju Patro (Ph.D. student), Department of Pharmaceutics, Al-Ameen College of Pharmacy, Bangalore, India, for helping us in the preparation of SZ and placebo gels. The authors report no conflicts of interest related to this study.

References

Agarwal E, Pradeep AR, Bajaj P and Naik SB. Efficacy of local drug delivery of 0.5 % clarithromycin gel as an adjunct to nonsurgical periodontal therapy in the treatment of current smokers with chronic periodontitis - A randomized controlled clinical trial. *Journal of Periodontology* 2012; **83:**1155-1163.

American Diabetes Association. Position statement. Standards of medical care in diabetes—2011. *Diabetes Care* 2011; **34**:s11.

Arrieta-Blanco JJ, Bartolome-Villar B, Jimenez-Martinez E, Saavedra-Vallejo P and Arrieta-Blanco FJ. Dental problems in patients with diabetes mellitus (II): Gingival index and periodontal disease. *Medicina Oral Patologia Oral Y Cirguia Bucal* 2003; **8**:233-247.

Bansal K, Rawat MK, Jain A, Rajput A, Chaturvedi TP and Singh S. Development of satranidazole mucoadhesive gel for the treatment of periodontitis. *American Association of Pharmaceutical Scientists Pharm Sci Tech* 2009; **10**:716-723.

Campus G, Salem A, Uzzau S, Baldoni E and Tonolo G. Diabetes and periodontal disease: A case-control study. *Journal of Periodontology* 2005; **76**:418-425.

Emrich L, Shlossman M and Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. *Journal of Periodontology* 1991; **62**:123-131.

Glavind L and Löe H. Errors in the clinical assessment of periodontal destruction. *Journal of Periodontal Research* 1967; **2**:180-184.

Gordon JM and Walker CB. Current status of systemic antibiotic usage in destructive periodontal disease. *Journal of Periodontology* 1993; **64**:760-771.

Grossi SG. Treatment of periodontal disease and control of diabetes: An assessment of the evidence and need for future research. *Annals of Periodontology* 2001; **6**:138-145.

Jansson H, Lindholm E, Lindh C, Groop L and Bratthall G. Type 2 diabetes and risk for periodontal disease: A role for dental health awareness. *Journal of Clinical Periodontology* 2006; **33**:408-414.

Jorgensen MG and Slots J. Responsible use of antimicrobials in periodontics. *Journal of the California Dental Association* 2000; **28**:185-193.

Kinane DF and Radvar M. A six-month comparison of three periodontal local antimicrobial therapies in persistent periodontal pockets. *Journal of Periodontology* 1999; **70**:1-7.

Kiran M, Arpak N, Unsal E and Erdogan MF. The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. *Journal of Clinical Periodontology* 2005; **32**:266-272.

Kornman KS and Löe H. The role of local factors in the etiology of periodontal disease. *Periodontology 2000* 1993; **2**:83-97.

Löe H. The gingival index, the plaque index and the retention index systems. *Journal of Periodontology* 1967; **38**:610-616.

Löe H. Periodontal disease. The sixth complication of diabetes mellitus. *Diabetes Care* 1993; **16**:329-334.

Mealy B. Diabetes mellitus. In Greenberg, M.S., Glick, M. (Eds): Burket's Oral Medicine Diagnosis and Treatment, 10th ed. New York. B.C. Decker, 2003; 563-577.

Nair MD and Nagarajan K. Nitroimidazoles as chemotherapeutic agents. *Progress in Drug Research*. 1983; **27**:163–252.

Nelson R, Shlossman M, Budding L, *et al.* Periodontal disease and NIDDM in Pima Indians. *Diabetes Care* 1990; **13**:836-840.

Pradeep AR, Sagar SV and Daisy H. Clinical and microbiologic effects of subgingivally delivered 0.5% azithromycin in the treatment of chronic periodontitis. *Journal of Periodontology* 2008; **79**:2125-2135.

Pradeep AR, Kalra N, Priyanka N, Khaneja E, Naik SB and Singh SP. Systemic ornidazole as an adjunct to non-surgical periodontal therapy in the treatment of chronic periodontitis: a randomized, double-masked placebo-controlled clinical trial. *Journal of Periodontology* 2012; **83**:1149-1154.

Pritchard JR and Higgins TJ. Tinidazole in clinical periodontics. The effects upon the subgingival microflora. *Journal of Dental Research* 1987; **66** (special issue):821 (Abst).

Seppala B, Seppala M and Ainamo J. A longitudinal study on insulin-dependent diabetes mellitus and periodontal disease. *Journal of Clinical Periodontology* 1993; **20**:161-165.

Steinberg D, Friedman M, Soskolne A and Sela MN. A new degradable controlled release device for treatment of periodontal disease: *In vitro* release study. *Journal of Periodontology* 1990; **61**:393-398.

Taylor GW. The effects of periodontal treatment on diabetes. *Journal of the American Dental Association* 2003; **134**:41-48.

Taylor GW and Borgnakke WS. Periodontal disease: Associations with diabetes, glycemic control and complications. *Oral Diseases* 2008; **14**:191-203.