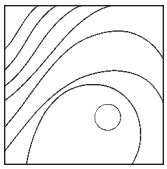
Clinical Efficacy of Subgingivally Delivered 1.2 mg Simvastatin in the Treatment of Patients with Aggressive Periodontitis: A Randomized Controlled Clinical Trial



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Simvastatin (SMV) is a specific competitive inhibitor of 3-hydroxy-2-methyl-glutaryl coenzyme A reductase that promotes bone formation. The present clinical trial was designed to investigate the effectiveness of 1.2 mg SMV as a local drug delivery system and as an adjunct to scaling and root planing (SRP) in the treatment of aggressive periodontitis (AgP). A total of 68 intrabony defects from 24 patients with AgP were treated either with 1.2 mg SMV gel or placebo gel. The subjects were randomly assigned to SRP + placebo (group 1; n = 12) or SRP + SMV (group 2; n = 12). Clinical parameters were recorded at baseline and at 3 and 6 months and included bleeding index, Plaque Index, probing depth (PD), and clinical attachment level (CAL). At baseline and after 6 months, radiologic assessment of bone defect fill was done. The mean decrease in PD at 6 months was 1.14 ± 0.04 mm and 3.78 ± 0.62 mm in groups 1 and 2, respectively. Significant gain in mean CAL was found between the groups (P < .05). Furthermore, significantly greater mean percentage of bone fill was found in group 2 (34.01%) compared to group 1 (2.62%). Locally delivered SMV provides a comfortable method to improve clinical parameters and promotes bone formation. Int J Periodontics Restorative Dent 2017;37:e135–e141. doi: 10.11607/prd.2936

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Aggressive periodontitis (AgP) is characterized by rapid loss of alveolar bone and consequent tooth loss, especially in the first molars and anterior incisors in otherwise healthy patients.[1] Generalized AgP is characterized by a pronounced episodic and rapid destruction of periodontal tissues, which may result in early tooth loss and shows an inadequate host response to periodontopathogenic bacteria caused by an increased expression of a wide variety of immunologic and genetic risk factors.[2]

Use of pharmacologic compounds that promote synthesis of osteogenic growth factors stimulate local bone formation and are a promising approach in the treatment of bone defects.

Statins are widely used to lower blood cholesterol levels. Some studies have reported that statins can stimulate bone formation by stimulating the production of bone morphogenetic protein-2.[3,4] Statins target the liver and have reduced affinity for bone tissue; in addition, orally administered statins are poorly distributed to bone.[5] The doses required for statins to have an effect on bone are much higher than those required to reduce cholesterol levels and are associated with unacceptable toxicity.[5] The primary advantage of local drug delivery is that smaller doses of topical

agents can be delivered inside the pocket, avoiding the side effects of systemic antibacterial agents while increasing the exposure to target microorganisms by reaching higher concentrations of the locally delivered drug, thus providing higher therapeutic levels of the medication.[6]

Simvastatin (SMV) is a member of the statin family, an inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, which is widely used as cholesterol-lowering drug. In addition, SMV suppresses the synthesis of mevalonate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate,[7] which in turn inhibits the formation and activity of osteoclasts.[8,9] Recently, our study showed that locally delivered 1.2 mg SMV stimulated a significant increase in probing depth (PD) reduction, clinical attachment level (CAL) gain, and improved bone fill as compared with placebo gel as an adjunct to scaling and root planing (SRP) in the treatment of intrabony defects (IBD) in chronic periodontitis patients[10] and in treatment of Class II furcation defects.[11]

To the best of the present authors' knowledge, there are no published data on the use of in situ gel using SMV with methylcellulose (as a vehicle) for direct placement in patients with AgP. Keeping the above facts in mind, the present study was carried out as a single-center, randomized controlled clinical trial to investigate the clinical and radiologic (bone fill) efficacy of 1.2 mg SMV, as an adjunct to SRP in the treatment of AgP compared to placebo gel.

Materials and Methods

Source of Data

The patients for this study were selected from the outpatient section of the Department of Periodontics, Government Dental College and Research Institute, from April 2012 to October 2012. A total of 24 patients aged 30 to 50 years (14 men and 10 women) who were diagnosed with AgP were enrolled in this study. It was made clear to the potential patients that participation was voluntary. Written informed consent was obtained from the patients, and ethical clearance for the study was received from the Institutional Ethical Committee and Review Board, Government Dental College and Research Institute, Bangalore.

Selection Criteria

The diagnosis of generalized AgP was based on the 1999 Consensus Classification of Periodontal Diseases.[12] Diagnostic criteria taken into consideration were only clinical, and not laboratory findings. Patients were classified as having AgP when there was evidence of the following characteristics: healthy status, other than the presence of periodontitis; rapid attachment loss and bone destruction, proven by radiographs obtained some years apart; familial aggregation; and clinical and radiographic diagnosis. Patients were diagnosed with generalized AgP when they presented with generalized interproximal PD and CAL of ≥ 5 mm and radiographic bone loss of ≥ 30% of root length affecting ≥ 3 permanent teeth other than first molars and incisors. Patients with no history of periodontal therapy or use of antibiotics in the preceding 6 months were included. Patients with known systemic disease or known or suspected allergy to the SMV group, those on systemic SMV therapy, those who used tobacco in any form, alcoholics, immunocompromised patients, and pregnant or lactating patients were excluded from the study.

A total of 26 patients were initially analyzed for the study. Two patients were excluded because they did not meet the inclusion criteria. After patient enrolment by an examiner (A.R.P.), the patients were randomly (by a computer-generated system) assigned to either the placebo group (group 1; n= 12) or SMV group (group 2; n = 12). In group 2, 33 sites were treated with SRP followed by 1.2 mg SMV gel (1.2 mg/0.1 mL) local drug delivery. In group 1, 35 sites were treated with SRP followed by placebo gel placement. For each patient, all selected sites (only molars) were treated with SMV or placebo gel based on the group to which they were allocated. Patients were masked for allocation to the two different groups. SRP was performed at baseline until the root surface was considered smooth and clean by the operator (P.N.). No antibiotics or anti-inflammatory agents were prescribed after treatment. Clinical parameters, including modified sulcus bleeding index (mSBI)[13] and full-mouth and site-specific Plaque Index (PI),[14] were considered. PD and CAL were recorded

at baseline (before SRP) and at 3 and 6 months. A North Carolina no. 15 color-coded periodontal probe (Hu-Friedy) was used to standardize the measurement of PD and CAL. A single clinician (P.N.) provided treatment to both groups. All pre- and post-treatment clinical parameters were recorded by another examiner (A.R.P.), who was masked to the type of treatment received (Fig 1).

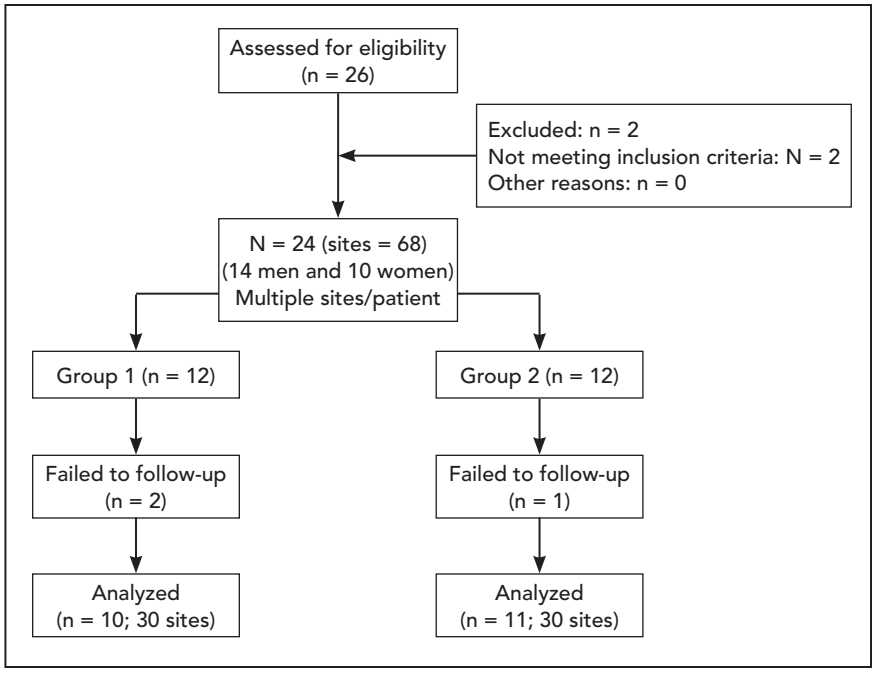

Fig 1 *Study flow chart.*

Radiographic Evaluation of IBD

Bone fill was evaluated at baseline and after 6 months using an image analyzer (Scion Image Analyzer, Scion) The radiographic bone fill was measured with a computer program according to the method used by Francetti et al.[15] Individually customized bite blocks and a parallel-angle technique were used to obtain films as reproducible as possible. All radiographs were reviewed in a single reference center by a masked evaluator. For evaluation, radiographs were scanned at 800 dots per inch with a scanner (HP Scanjet 3c/I, Hewlett Packard) and bone defect was evaluated using computer software. IBD was determined on the radiograph by measuring the vertical distance from the crest of the alveolar bone to the base of the defect.

Intraexaminer Calibration

Intraexaminer calibration was achieved by examining 20 patients twice 24 hours apart before beginning the study. Measurements at baseline and at 24 hours fell within the limit of 1 mm of change at 95% confidence level.

Primary and Secondary Outcome Measures

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

Formulation of 1.2 mg SMV In Situ Gel

The SMV gel (1.2 mg) was prepared as described in a previous study by the present group.[10] Briefly, methylcellulose in situ gel was prepared by adding the required amount of biocompatible solvent to an accurately weighed amount of methylcellulose. The vial was heated to 50°C to 60°C and agitated using a mechanical shaker to obtain a clear solution.[16] A weighed amount of SMV was added to this solution and dissolved completely to obtain a homogenous phase of polymer, solvent, and drug. Thus, the SMV in situ gel was prepared with a concentration of ~1.2 mg.

Local Drug Delivery

For standardization, 0.1 mL prepared SMV gel (1.2 mg/0.1 mL) was injected into the IBD using a syringe with a blunt cannula. After placement of the in situ gel, patients were

Table 1 PD, CAL, and mSBI for Groups 1 and 2 (mean ± SD) at Different Time Intervals

Group	PD (mm)	*P*	CAL (mm)	*P*	mSBI	*P*
1						
Baseline	6.70 ± 1.21		7.22 ± 1.42		2.86 ± 0.46	
3 mo	5.12 ± 1.33	.001*	6.13 ± 0.87	.001*	2.39 ± 0.27	.001*
6 mo	5.56 ± 1.25	.001*	5.86 ± 0.62	.001*	2.56 ± 0.15	.001*
2						
Baseline	6.93 ± 1.37		7.85 ± 1.45		2.79 ± 0.28	
3 mo	4.11 ± 0.83	.001*	4.17 ± 1.23	.001*	1.22 ± 0.94	.001*
6 mo	3.15 ± 0.75	.001*	3.99 ± 1.04	.001*	1.25 ± 0.19	.001*

*Statistically significant at 5% level of significance ($P < .05$).

Table 2 Site-Specific PI for Groups 1 and 2 at Different Time Intervals

	Baseline		3 mo		6 mo	
PI	Group 2 n (%)	Group 1 n (%)	Group 2 n (%)	Group 1 n (%)	Group 2 n (%)	Group 1 n (%)
0	0	0	22 (73.3)	20 (66.6)	26 (86.6)	24 (80.0)
1	08 (26.6)	07 (23.3)	8 (26.6)	10 (33.3)	04 (13.3)	06 (20.0)
2	10 (33.3)	12 (40.0)	0	0	0	0
3	12 (40.0)	11 (36.6)	0	0	0	0
*P**	NS	NS	NS	NS	NS	NS

*Not statistically significant at .05 level of significance.
NS = not significant.

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.

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. The categoric variable (site-specific PI) was expressed as a percentage and continuous variables (full-mouth PI, mSBI, PD, CAL, and bone fill) as mean ± standard deviation (SD). Site-specific PI was compared using chi-square test or Fisher exact test when the expected frequency was < 5. Normality assumption was tested using Shapiro-Wilk W test. Between the treatment groups, comparison was carried out using Mann-Whitney test. The adjusted mean at each visit is shown in Table 1. Wilcoxon signed rank test was used for comparison within groups. Statistical significance was defined as $P < .05$. Statistical analysis was performed with statistical software (SPSS version 10.5, SPSS).

Results

Of the 24 patients enrolled, 21 completed the study. Of these, 2 patients did not follow up after the baseline examination and 1 patient refused to participate because of reasons unrelated to the study. A total of 60 treatment sites (multiple sites/patient) were evaluated for clinical parameters at baseline (before SRP) and at 3 and 6 months. Radiographic parameters were recorded at baseline and at 6 months in 60 treatment sites.

Clinical Evaluation

No adverse reaction was observed in any patient from group 2, and no patient reported any discomfort. Healing was uneventful. All patients tolerated the drug with no post-application complications.

Evaluation of Oral Hygiene

No statistically significant difference was found between groups 1 and 2 at any time for full-mouth PI or for PI at the test site (Tables 2 and 3). This indicates that both groups maintained comparable levels of oral hygiene throughout the study.

mSBI

A statistically significant decrease in mSBI scores from baseline was found in both groups. The decrease was greater in group 2 (1.54 ± 0.09 mm) compared with group 1 (0.30 ± 0.31 mm) at 6 months (Table 1).

Table 3 Full-Mouth PI for Groups 1 and 2 at Different Time Intervals

Group	PI (mean ± SD)	*P*
1		
Baseline	1.87 ± 0.22	
3 mo	0.81 ± 0.16	NS
6 mo	0.66 ± 0.13	NS
2		
Baseline	1.98 ± 0.24	
3 mo	0.92 ± 0.32	NS
6 mo	0.75 ± 0.21	NS

NS = not significant.

Table 4 Comparison of IBD Values from Baseline to 6 Months

Group	IBD (mm) (mean ± SD)	IBD decrease (mm) (mean ± SD)	IBD decrease (%)	*P*
1				
Baseline 6 mo	4.57 ± 0.86 4.45 ± 0.72	0.12 ± 0.14	2.62	NS
2				
Baseline 6 mo	4.88 ± 1.22 3.22 ± 0.37	1.66 ± 0.85	34.01	.001*

*Statistically significant.

PD

The decrease in PD was statistically significant within both groups compared to baseline at all time intervals (Table 1). However, group 2 showed significantly greater PD reduction at 3 and 6 months than group 1, at $P < .001$.

CAL

The difference 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 (Table 1).

Bone Fill

There was a greater bone fill in group 2 (34.01%) compared to group 1 (2.62%), and it was statistically significant ($P < .001$) (Table 4).

Discussion

The current study evaluated the clinical efficacy of 1.2 mg SMV in situ gel as an adjunct to SRP for treatment of IBD in patients with AgP and showed significant radiographic bone fill and improvement in clinical parameters compared with placebo gel.

To the authors' knowledge, there have been no studies reporting the use of 1.2 mg SMV gel as local drug delivery in the treatment of patients with AgP. Comparing change in clinical and radiographic parameters with 1.2 mg SMV gel as local drug delivery used in the treatment of patients with chronic periodontitis in a previous study,[10] greater improvement in clinical parameters was found in patients with chronic periodontitis, whereas greater radiographic bone fill was observed in patients with AgP in the current study. PD reduction was greater in patients with chronic periodontitis (4.26 ± 1.59 mm) as reported in a previous study[10] compared to patients with AgP in the current study (3.78 ± 0.62 mm) at 6 months. CAL gain also was greater in patients with chronic periodontitis (4.36 ± 1.92 mm)[10] compared to patients with AgP in the current study (3.86 ± 0.41 mm) at 6 months. Conversely, bone fill percentage was nearly equal but greater in patients with AgP (34.01%) compared to patients with chronic periodontitis (32.54%).[10]

A number of studies have concentrated on the effects of locally administered SMV on bone formation.[10,17] The local tissue concentration of a drug can be enhanced by incorporating the active agent into controlled-release delivery systems to be placed directly in the periodontal pocket or the defect area. The present study has reported locally delivered 1.2 mg SMV to be highly effective in promoting bone formation in IBD in patients with chronic periodontitis[10,18] and Class II furcation defects.[11]

Sakoda et al[19] measured the effect of SMV on interleukin (IL)-6 and -8 production in a cultured human epithelial cell line (KB cells) in response to IL-1. SMV decreased the production of IL-6 and IL-8, an effect that was reversed by adding mevalonate or geranyl pyrophosphate, but not farnesyl pyrophosphate. SMV reduced nuclear factor-kappa B and activator protein 1 promoter activity in KB cells, indicating an anti-inflammatory effect for SMV on human oral epithelial cells, apparently involving Rac1 GTPase (a hydrolase enzyme that can bind and hydrolyze guanosine triphosphate) inhibition. At a low concentration, SMV exhibits a positive effect on the proliferation and osteoblastic differentiation of human periodontal ligament cells. These effects may be caused by inhibition of the mevalonate pathway. SMV is reported to stimulate vascular endothelial growth factor (VEGF) release in a dose-dependent manner, and the authors suggested that statins may promote osteoblast differentiation and bone nodule formation by stimulating VEGF expression in bone tissue.[20]

In the present study, bleeding index decreased from baseline to 6 months, suggesting an anti-inflammatory effect of SMV. A similar anti-inflammatory effect was observed in the present authors' previous study[10] in patients with periodontal IBD. Statins reduce the plasma levels of inflammatory markers such as C-reactive protein (CRP).[21] A statin-mediated decrease in CRP concentrations could be due to inhibition of IL-6 in the vascular tissues.[22] Thus, statins, including SMV, are believed to have biologically significant antioxidant and anti-inflammatory effects, which could prove beneficial in the treatment of periodontitis.

A significant mean decrease in IBD from baseline to 6 months in group 2 suggests a role for SMV in bone formation in AgP. This may be because of increased bone morphogenetic protein-2 expression during bone regeneration,[23] anti-inflammatory effects,[24] and angiogenesis during wound healing.

With regard to the dose of SMV used in the present study, 1.2 mg/0.1 mL per site was injected. It has been shown that local application of approximately 70 mg/kg causes inflammation.[16] Stein et al[25] demonstrated reducing the SMV dose from 2.2 to 0.5 mg decreased inflammation to a more clinically acceptable level without sacrificing bone growth potential. However, inadequate dosing of SMV results in lack of bone augmentation in intrabony and furcation defects. Inadequate dosing may be the result of gel extrusion from the defects, since it is difficult to achieve proper viscosity with a lower concentration. However, 1.2 mg SMV has proven to be the optimal dose in IBD.[10,18]

The present study, in which only a nonsurgical approach (SRP) was used in conjunction with locally delivered 1.2 mg SMV, effective bone fill and a greater decrease in PD and CAL gain were found in the treatment of IBD. Therefore, this study confirms that locally delivered SMV promotes bone growth in IBD in patients with AgP.

Conclusions

This clinical trial demonstrates that local delivery of 1.2 mg SMV into the periodontal pocket in group 2 patients stimulated a significant increase in PD reduction and CAL gain, and improved bone fill compared to group 1 patients. However, long-term, multicenter, longitudinal studies using different vehicles and concentrations of SMV should be carried out to affirm the observations of this study.

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