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Research Article

A SPLIT MOUTH STUDY DESIGN SHOWING CLINICAL EFFICACY OF SUBGINGIVALLY DELIVERED 1.2% ATORVASTATIN IN CHRONIC PERIODONTITIS

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ABSTRACT

Background: Atorvastatin (ATV) is a specific competitive inhibitor of 3-hydroxy-2-methyl-glutaryl coenzyme A reductase. Recently, statins have shown pleiotropic effects like antiinflammation and bone stimulation. The aim of the present study was to evaluate the clinical efficacy of subgingivally delivered 1.2% controlled release Atorvastatin gel as an adjunct to scaling and root planing in chronic periodontitis patients.

Methods: Thirty-four sites in seventeen patients were categorized into two treatment groups: SRP only (group A) and SRP plus subgingival application of 1.2% Atorvastatin gel (group B). Clinical parameters were recorded at baseline before SRP and at 1 and 3 months; they included plaque index (PI), gingival index (GI) probing pocket depth (PPD), relative attachment level (RAL)

Results: Mean probing depth reduction and mean clinical attachment level gain was found to greater in ATV group as compared to group A, at 1 and 3 months.The decrease in GI score at 3 months was greater in group B (1.058±0.300) compared to group A (1.426±0.361). The mean decrease in PPD from baseline to 3 months was 5.235±1.601 and 3.647±1.934 in groups A and B, respectively. Mean RAL gain from baseline to 3 months was7.823±2.455 and 6.647±2.596 in groups A and B, respectively.

Conclusion: There was a greater decrease in gingival index and PPD and more RAL gain at sites treated with SRP plus locally delivered ATV in patients with chronic periodontitis.

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INTRODUCTION

Periodontal disease results from the interaction of the host defence mechanisms with plaque micro organisms.[1] Though micro-organisms have been implicated as the etiological factor for periodontal disease, pathology of these inflammatory lesions have been attributed not only to bacterial products, but also to chemical mediators released by the host cells, as a result of inflammatory and immune reactions.

Various vehicles, such as lactide/glycolide polymer,[2,3] hydroxypropyl methylcellulose,[4] and carbopol,[5,6] have been mentioned and used in the literature for controlled-drug release.[SCP]

Local delivery of chemotherapeutic agents into the pockets via a syringe or irrigating device has been shown to be clinically efficacious.[7,8] Different drugs used for local delivery are tetracyclines including doxycycline and minocycline, metronidazole and chlorhexidine, growth factors and certain novel herbal and therapeutic agents, statins being one amongst them.[9] Methylcellulose is widely used in a variety of oral and topical pharmaceutical formulations, such as ophthalmic controlled-release in situ gelling systems for ciprofloxacin and nimesulide- loaded methylcellulose nanoparticles and microparticles for oral delivery.[10,11] It is used extensively in cosmetic and food products. Methylcellulose is generally regarded as a non-toxic, non-allergic, and non-irritating material and is used as a sustained released vehicle for therapeutic drugs.[12]

Statins like Simvastatin (SMV), Atorvastatin (ATV) and lovastatin are specific competitive inhibitors of 3-hydroxy-2-methyl-glutaryl coenzyme A (HMG-CoA) reductase, used to lower cholesterol provide an important and effective approach for the treatment of hyperlipidemia and arteriosclerosis.[13-15] In addition, statins have been found to exert anti-inflammatory and immunomodulatory actions.[16] Statins also seem to modulate bone formation by increasing the expression of bone morphogenetic protein-2 (BMP-2), thus providing a new direction in the field of periodontal therapy.[17] Patients on statin medication exhibit fewer signs of periodontal inflammatory injury than subjects without the statin regimen.[18]

In addition, it has also been suggested that statins directly affect osteoclasts through

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mechanisms analogous to those of bisphosphonates, because bisphosphonates and statins exert their effects by inhibiting the same mevalonate pathway.[19] Sakoda *et al*. measured the effect of SMV on interleukin (IL)-6 and -8 productions in a cultured human epithelial cell line (KB cells) in response to IL-1. SMV was found to reduce NF-κB and AP-1 promoter activity in KB cells.[20]

ATV has been found to be more effective when compared to SMV and pravastatin in patientswith hyperlipidemia.[21] In a study ATV therapy decreased tumor necrosis factor (TNF)-α production in lipopolysaccharides (LPS) activated monocytes, confirming the anti-inflammatory properties of this class of drugs.[22]Moreover treatment of human osteoblasts with ATV enhanced the expression of osteoblastic differentiation markers like alkaline phosphatase and osteocalcin.[23]

Recently a study has shown ATV might have beneficial effects on alveolar bone loss and tooth mobility in individuals with periodontal disease.[24] Goes *et al*. have shown that ATV was able to prevent alveolar bone loss seen on a ligature-induced periodontitis in Wistar rats.[25] Keeping the above facts in mind, the present study was carried out to assess and compare the clinical efficacy of 1.2 % ATV gel as an adjunct to SRP in the treatment of chronic periodontitis.

MATERIALS AND METHODS

Source of Data

The patients for this study were selected from the outpatient section of the Department of Periodontics and Oral Implantology, M.M College of Dental Sciences and Research, Mullana, Ambala.

Thirty-four sites in seventeen patients, aged 25 to 48 years (either males and females) who were diagnosed with chronic periodontitis, were enrolled in this study. The study was conducted as per the Declaration of Helsinki *(1964 revised in 2008),* with the approval of Institutional Ethical Committee of Maharishi Markandeshwar University. The subjects for the study were selected from amongst the outpatient department and each patient was given detailed verbal and written description of risks and benefits of treatment with the consent to treatment agreement.

Selection Criteria

Systemically healthy individuals with periodontal pockets ≥5mm or relative attachment level (RAL) ≥ 4 mm and no history of antibiotic or periodontal therapy in the preceding 6 months were included. Individuals with known or suspected allergy to the ATV/statin group, those on systemic ATV/statin therapy, individuals with aggressive periodontitis, use of tobacco in any form, alcoholics, individuals with diabetes, immunocompromised individuals, and pregnant or lactating females were excluded.

After the subject selection, thirty-four sites in seventeen patients , atleast two sites, one in each quadrant or contralateral sides of the arch were divided into two groups as Group A sites and Group B sites using split mouth study design.[26,27] with a radiographic evidence of bone loss.(Fig:1) Group A sites were treated with scaling and root planing (SRP) only while Group B sites were treated with scaling and root planing along with Intracrevicular application of 1.2% Atorvastatin gel (1.2 mg/0.1ml)

SRP was performed at baseline until the root surface was considered smooth and clean by the operator. No antibiotics or antiplaque and anti-inflammatory agents were prescribed after treatment. Clinical parameters, including gingival index (GI) plaque index (PI), probing pocket depth (PPD) and relative attachment level (RAL) were recorded at baseline, one month and three months post operatively. The Plaque index by **Silness and Loe 1964**[28] was included in the study as it reflects the oral hygiene status of the patient throughout the study. The **Loe and Silness 1963**[29] Gingival index was used to visually score gingivitis on papillae and margins of facial and lingual gingivae of natural teeth. The Probing pocket depth and Relative attachment level were recorded using UNC-15 probe (Guentsch A *et al* 2008)[30] and occlusal stent as a reference point (Clark DC *et al* 1987).[31]

Formulation of 1.2 % ATV Gel

After intensive in vitro investigations for optimization and stability, the ATV gel was developed at the M.M College of Pharmacy, Mullana, Ambala. Methylcellulose in situ gel was prepared as described by Thylin *et al*.[32] 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. A weighed amount of ATV was added to the above solution and dissolved completely to obtain a homogeneous phase of polymer, solvent, and drug. Thus, the ATV in situ gel was prepared with a concentration ~1.2%.

Local Drug Delivery

For standardization, 10 µl prepared ATV gel (1.2 mg/0.1 ml) was loaded into the disposable syringe with an angulated blunt tip. The tip of the needle was slowly slided over the tooth surface till the base of the pocket, and then the gel was injected slowly till the pocket was overfilled (Fig:2). Excess gel was removed and the subjects were instructed to refrain from drinking, eating 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, 34 sites in 17 patients were required. All the preoperative and postoperative values were subjected to statistical analysis. The arithmetic mean and standard deviations were calculated for the requisite assessment intervals. '**Wilcoxon Signed Rank Test**' was performed for intra-group variations. **'Independent t-Test'** was performed for comparison of inter-group variations in clinical parameters. Statistical significance was defined as $p<0.05$.

Ex vivo Assessment of drug Retention and in vitro drug Permeation of Prepared gel

The drug permeation and drug retention pattern assessment was carried out in the College of Pharmacy, Maharishi

Markandeshwar University, Mullana. The drug retention was assessed on alveolar mucosa taken from the extraction sites of the patients. Franz diffusion Cell method was used to estimate the drug retention. The assay for drug permeability was analysed on UV-visible spectrophotometer.

RESULTS

Possible side effects of therapy including slight discomfort and gingival redness were evaluated. No treatment related adverse effects were observed in any patient. **Garret *et al* 1999**[33] also reported that emergent adverse events constituted ≤ 1% of the entire study population with 0.2% of them showing allergic response.

Fig 1 Radiographic evidence of bone loss

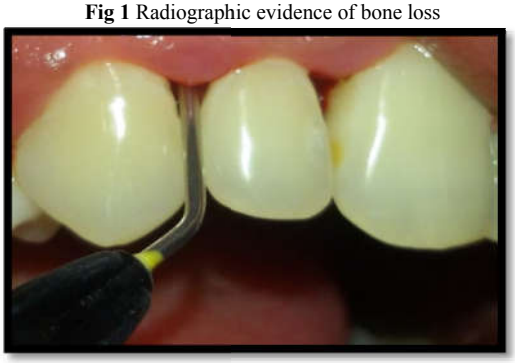


Fig 2 Subgingival application of 1.2 % Atorvastatin Gel

Table 1 Mean and Mean difference in Plaque index and Gingival index of Group A and B at different intervals

		PLAQUE INDEX				GINGIVAL INDEX			
	Assessment Interval	Mean±SD	Mean Difference From Baseline	Z Value	P Value	Mean±SD	Mean Difference From Baseline	Z Value	P Value
GROUP A	Baseline	1.764±0.437				1.882±0.523			
	1 month	1.397±0.331	0.367±0.332	-3.042	.002	1.426±0.361	0.455±0.321	-3.325	.001
	3 months	1.426±0.339	0.338±0.341	-2.901	.004	1.426±0.361	0.455±0.321	-3.325	.001
GROUP B	Baseline	1.735±0.471				1.852±0.523			
	1 month	1.029±0.277	0.705±0.377	-3.455	.001**	1.058±0.300	0.794±0.435	-3.482	<.001**
	3 months	1.088±0.330	0.647±0.415	-3.359	.001**	1.058±0.300	0.794±0.435	-3.482	<.001**

Table 2 Mean and Mean difference in Pocket probing depth and Relative attachment level of Group A and B at different intervals

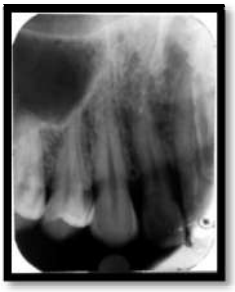
		Pocket probing depth				Relative attachment level			
	Assessment Interval	Mean±SD	Mean Difference From Baseline	Z Value	P Value	Mean±SD	Mean Difference From Baseline	Z Value	P Value
GROUP A	Baseline	5.823±1.236				8.411±2.093			
	1 month	5.411±1.460	0.411±0.507	-2.646	.008	8.000±2.318	0.411±0.507	-2.646	.008
	3 months	5.235±1.601	0.588±0.618	-2.887	.004	7.823±2.455	0.588±0.618	-2.887	.004
GROUP B	Baseline	6.058±1.712				8.764±2.634			
	1 month	3.764±1.921	2.294±0.685	-3.695	<.001**	6.705±2.568	2.058±0.747	-3.741	<.001**
	3 months	3.647±1.934	2.411±0.618	-3.710	<.001**	6.647±2.596	2.117±0.696	-3.777	<.001**

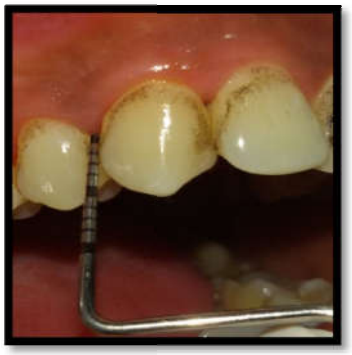
Table 3 Intergroup comparison of Mean differences in Plaque index and Gingival index at different intervals

		PLAQUE INDEX			GINGIVAL INDEX		
	Assessment Interval	Mean Difference (A-B)	t Value	P Value	Mean Difference (A-B)	t Value	P Value
GROUP A vs. B	Baseline						
	1 month	-0.338	-2.774	.009**	-0.338	-2.578	.015*
	3 months	-0.308	-2.368	.024	-0.338	-2.578	.015*

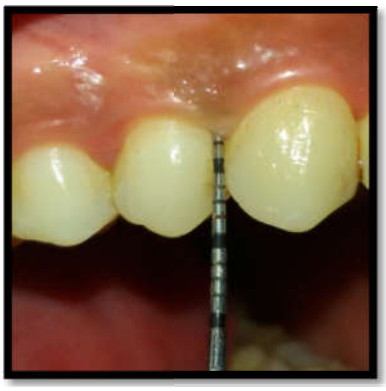
Table 4 Intergroup comparison of Mean differences in Pocket probing depth and Relative attachment level at different intervals

		Pocket probing depth			Relative attachment level		
	Assessment Interval	Mean Difference (A-B)	t Value	P Value	Mean Difference (A-B)	t Value	P Value
Group A vs. B	Baseline						
	1 month	-1.882	-9.097	<.001**	-1.647	-7.517	<.001**
	3 months	-1.823	-8.598	<.001**	-1.529	-6.770	<.001**





A



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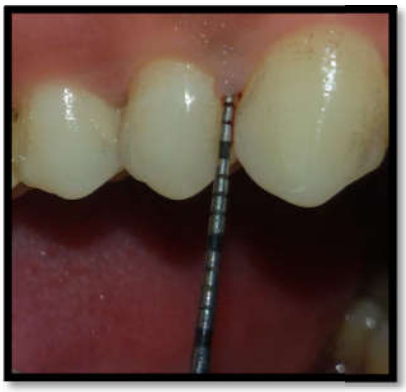

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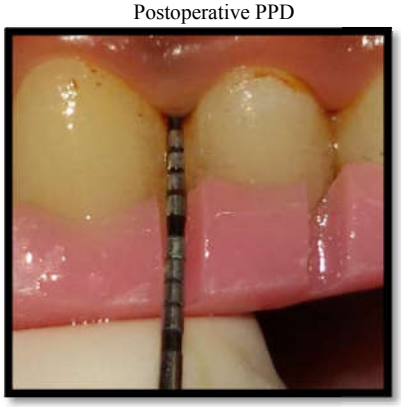
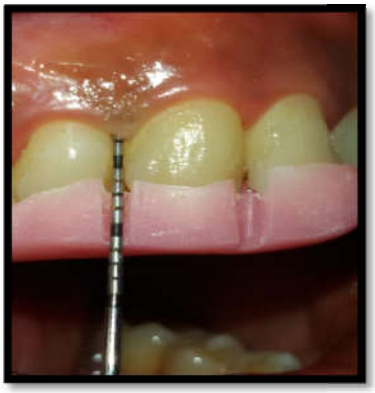
Figure 3 a- Preoperative PPD, b-1 month Postoperative PPD,c- 3- months Postoperative PPD



A



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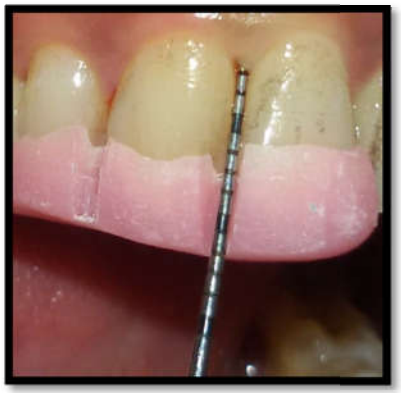

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Figure 4 a- Preoperative RAL, b-1 month Postoperative RAL, c- 3- months Postoperative RAL

DISCUSSION

Advantages of using the subgingival drug-delivery system include achieving high intrasulcular drug concentrations, avoiding its systemic side effects, and better patient compliance.[34,35] Therefore, this in vivo study using ATV in situ gel formulation was carried out to assess the benefits of a local drug-delivery system.

Fajardo *et al*. in a study found that ATV administration increased alveolar bone height, decreased the CEJ to alveolar bone distance and had beneficial effect on tooth mobility in individuals with periodontal disease over a period of 3 months.[24] Effects of statins on the periodontium are quite poorly understood. Saxlin *et al*. found dual effect of statin medication on the periodontium and showed that individuals with no gingival bleeding, statin medication was found to be associated with an increased likelihood of having deepened periodontal pockets.[36] Previously Morris *et al*. studied the effect of injectable SMV in three walled periodontal IBDs, Class 2 furcations defects, and edentulous alveolar ridges in beagle dogs by histomorphometric analysis, 29% greater ridge thickness was found with SMV, but bone height loss was detected in the interproximal intrabony and furcation defects.[37] In the present study there was a significant decrease in GI index from baseline to 3 months, suggesting an anti-inflammatory effect of ATV. A similar anti-inflammatory effect of statin was observed by Lindy *et al*.[18] in patients with chronic periodontitis who were on systemic statin therapy. Patients with periodontitis taking statins had 37% fewer pathologic periodontal pockets than those not taking statin medication.

Recently, ATV has been reported to inhibit inflammatory cells and matrix metalloproteinases (MMPs), which play a role in the connective tissue destruction in periodontal disease.[38] MMPs levels have been reported to be highly correlated to PD and bleeding on probing.[39] Similarly SMV has been found to show the anti-inflammatory effect on human oral epithelial cells, apparently involving Rac1 GTPase inhibition and decreased IL-6 and IL-8 production.[20] In a study, 8-week treatment with ATV reduced CD36 expression and decreased nuclear NFκB levels which is known to be involved in TNF-α production, in circulating monocytes from patients with type 2 diabetes.[22] CD36 is a class B family scavenger receptor which is able to bind Ox-LDL, is up-regulated in response to oxidative stimuli, such as oxidatively modified low-density lipoprotein (Ox-LDL) itself and IL-4 and by a signaling pathway involving protein kinase C.[40] OxLDL has been found in the gingival crevicular fluid and has shown to enhances IL-8 production in epithelial cells.[41] Since NFκB activation seems to be part of the stress response to oxidative stimuli, the reduction of the nuclear-active and the increase of the cytosolic-inactive NFκB form observed in these patients, after ATV treatment, might indicate reduced oxidative stress.[22]

Moreover, Majima *et al*. suggested beneficial effect of ATV, on bone metabolism by reducing bone resorption rather than by stimulating bone formation in hypercholesterolaemic patients treated for 3 months.[42] Additionally, ATV has been reported to increase numbers of circulating endothelial progenitor cells.[43]ATV increases the secretion of OPG, a potent inhibitor of bone resorption in human osteoblasts.[23] Second, statins

directly affect osteoclasts through mechanisms, which closely resemble the mode of action of nitrogen-containing bisphosphonates and a third paracrine pathway, which acts through osteoblast-osteoclast cross talks and involves the RANKL/OPG system.[19,23]

This study showed that there was statistically significant difference in PI and GI. However, a highly significant reduction in PPD (3.764±1.921 at 1month and 3.647±1.934 at 3 months) and gain in RAL(6.705±2.568 at 1 month and 6.647±2.596 at 3 months) was observed in Group B as compared to Group A ($p<0.001$). This was in accordance to the study by Pradeep AR *et al* (2010)[7] who showed significantly greater probing depth reduction and significant improvement in CAL for the use of simvastatin gel with SRP in comparison to the control group having SRP with placebo gel.
ATV is a lipophilic statin which appear to have a more potent bone-sparing effect as compared to hydrophilic statins such as pravastatin.[44,21,45]

The current study has considered the technique of subgingivally delivering ATV directly into peridontal pockets in individuals with chronic periodontitis as the LDD system offer the advantages of high concentrations at the target site with reduced dosage, fewer applications, and high patient acceptability.[46] Compared to a systemic regimen, local delivery may offer important benefits in terms of adverse reactions and patient compliance as it is also reported in previous study.[7,47]

Statins are effective drugs in the treatment of hyperlipidaemia and the prevention of cardiovascular events. However, despite the proven efficacy and safety of statins, relevant side effects exist and should be considered when treating patients. Statins are safe and have few side effects like muscle toxicity with myopathy and rhabdomyolysis, which is rare and mostly occurs in patients on higher statin doses and on drugs that interact at the level of hepatic metabolism.[48]

CONCLUSION

This clinical trial thus demonstrates that local delivery of 1.2% ATV(Group B) into the periodontal pockets in individuals with chronic periodontitis stimulated a highly significant reduction in Plaque index, Gingival index, Pocket probing depth and gain in Relative attachment level at 1 and 3 months from baseline as compared to Group A in adjunct to SRP. The overall observations of the present study were encouraging and showed promising results with significant improvement in all the clinical parameters. However, long-term studies, using different vehicles and concentrations of ATV, should be carried out to affirm the observations of our study.

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