Effectiveness of Simvastatin 1% oral gel and mouthwash used as an adjunct treatment of scaling and root planning in the treatment of periodontal diseases

Faiza Hasan[1]*, Rahila Ikram[2], Shabana Usman Simjee[3], Kanwal Iftikhar[3] and Kamran Asadullah[4]

[1]Department of Pharmacology, Fatima Jinnah Dental College, Karachi, Pakistan
[2]Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[3]HEJ Research Institute of Chemistry, International Centre of Chemical and Biological Sciences, University of Karachi, Pakistan
[4]Crown Dental Clinic, Karachi, Pakistan

Abstract: Simvastatin is an anti-hyperlipidemic drug which reduces the cholesterol synthesis and also has anti-inflammatory, immunomodulatory and anti-microbial action against the bacteria. This develops the interest of periodontologist to use it in combination with conventional treatment to treat periodontal diseases. The objective of the study was to develop the gel and mouthwash of simvastatin and use it locally to treat gingivitis and periodontitis as an adjunct to scaling and root planning. The patients were randomly allocated into three groups that were standard treatment group, gel treatment group and mouthwash treatment group. Results indicated that simvastatin gel and mouthwash in 1% preparation showed favorable results by significantly reducing periodontal parameters and inflammatory biomarkers ($p \leq 0.001$) as compared to standard treatment. Thus, we strongly suggest the use of simvastatin by local drug delivery system as an adjunct treatment of scaling and root planning.

Keywords: Simvastatin, scaling and root planning, gel, mouthwash, periodontal diseases.

INTRODUCTION

Simvastatin is an anti-hyperlipidemic drug which decreases the cellular cholesterol synthesis. It is an inhibitor of HMG CoA reductase enzyme and used for prophylaxis to reduce cardiovascular diseases (Kim *et al.,* 2018). It not only reduces the cholesterol synthesis but also have anti-inflammatory, immunomodulatory and anti-microbial action against the bacteria (Kaminska *et al.,* 2018) which develops the interest of periodontologist to used it in combination with conventional treatment to treat periodontal diseases.

The atherosclerotic process increases during periodontal diseases initiating humoral and cell mediated inflammation. A positive connection was found between periodontal diseases and CV diseases. This is due to *P. gingivalis* that moves in the aorta's endothelial cell and heart to promote the aggregation of platelets and formation of thrombus by releasing collagen and toxic substances. Modification in humoral and cell mediated response improves the severity and progression of inflammation by using an effective anti-inflammatory and anti-microbial agent. A very low value of MIC of simvastatin proved to be an effective anti-microbial agent with anti-inflammatory properties (Emani *et al.,* 2018).

Different researches are going on the local drug delivery system in dentistry since 1979. Researchers found out good results by the use of different drugs which are delivered locally to treat periodontal diseases. These local preparations applied in to the periodontal pockets with scaling and root planning because only scaling and root planning does not completely eradicate the bacteria from inaccessible sites. As a result, the bacteria regrow there and form a complex biofilm which may lead to re-infection. This drug delivery system requires less intervention, lessens the dose and sustains the drug's therapeutic level in the gingival crevicular fluid for long periods, causing minimum or no side effects (Agarwal *et al.,* 2016; Gunjiganur *et al.,* 2017).

The role of inflammatory mediators in gingivitis and periodontitis is well known. Now a day's saliva is used as an inflammatory biomarker in many inflammatory diseases including, periodontal diseases (Prasad *et al.,* 2016). Macrophages and gingival fibroblasts release TNF-α and IL-1β which are accountable for the production of prostaglandin E_2, IL-6 and IL-8 (Hasturk *et al.,* 2012). Nitric oxide levels are also imbalanced in gingivitis and periodontitis and the numbers of free radicals are increased during these inflammatory conditions (Gupta *et al.,* 2015).

The pro-inflammatory cytokines increases the activity of osteoclasts thus causing loss of alveolar bone and consequently attachment and tooth loss. In a study, oral use of simvastatin for consecutive 3 years showed reduction in alveolar bone loss (Cunha-Cruz *et al.,* 2005).

**Corresponding author:* e-mail: drfaiza77@hotmail.com

Simvastatin blocks the production of mevalonate which decrease the number of osteoclasts and production of IL-6. On the other hand, it increases alkaline phosphatase activity, endothelial growth factor and increases mineralization thus improves osteoblastic proliferation and differentiation of periodontal ligament cells. So the use of these low cost drugs in local formulations increases osteoblastic activity, produce bone growth factors and a very cheap alternative to treat bone defects (Agarwal *et al.,* 2016).

Thus, the objective of the study was to develop the gel and mouthwash of simvastatin and use it locally to treat gingivitis and periodontitis as an adjunct to scaling and root planning.

MATERIALS AND METHODS

Preparation of Simvastatin 1% gel and mouthwash

Tablets of 20 mg of simvastatin were used for 1% gel preparation. After dissolving the tablets in distilled water, the preparation was added in 1% carbapol gel containing preservatives. The pH of the final preparation was adjusted by adding triethanolamine solution. The viscosity, syringeability, spreadability, mucoadhesiveness and pH of the gel were evaluated before its application in to the patient's mouth. The microbial count was also done for quality control of the formulation (Ranjan *et al.,* 2017).

Similarly, 1% mouthwash was also prepared, using 20 mg tablets of simvastatin by dissolving them in distilled water. Sodium benzoate was included as preservative followed by glycerin and natural food color and flavor to be tolerated by mouth. The final preparation was then adjusted for its pH by adding triethanolamine solution. The quality of mouthwash was also assessed before its application.

Study design

Randomized clinical control trial

Data source

This study was done in the Pharmacology department of Karachi University, Sindh, Pakistan and HEJ Research Institute of Chemistry Karachi University, Sindh, Pakistan. The patients were enrolled during December, 2017 to March, 2018. The patients who were agreed to participate in the study signed the consent form. Complete medical and dental history of the patients was taken before starting the procedure.

Ethical approval

All methods included in this study involving human subjects were in accordance to the Helsinki declaration (2013). Approval of the studies was obtained from the board of advanced studies and the Independent Ethics committee of International Center for Chemical and Biological sciences ICCBS/IEC-029-HS2017/Protocol/ 1.0.

Patient's inclusion criteria

- Patients of gingivitis and periodontitis without any systemic diseases
- Adult patients of more than 18 years of age.

Patient's exclusion criteria

- Patients taking any medicines from six months
- Patients had periodontal treatment since last two months
- Pregnant females and lactating mothers

Patients group

Thirty patients were allocated randomly into three groups. Scaling and root planning was done in each mouth quadrant once a week for 4 consecutive weeks in each group.

Group A: Received only scaling and root planning.

Group B: Gel was applied in the treated quadrant after 48 hours of each session.

Group C: Mouthwash was used by the patients after the completion of each session.

Oral clinical parameters

The following six oral clinical parameters were observed

a) Probing depth (Ranjan *et al.,* 2017)
b) Clinical attachment level (Ranjan *et al.,* 2017)
c) Plaque and gingival index (Caygur *et al.,* 2017)
d) Tooth mobility (Zhang *et al.,* 2017)
e) Bleeding on probing (Pei *et al.,* 2017)

All the parameters were taken before and after 4 weeks of treatment.

Collection of saliva and analysis of salivary biomarkers

Standard protocol was used for the collection of unstimulated saliva. The saliva samples were refrigerated after centrifugation. PGE_2 (Zhang *et al.,* 2017), TNF- α (Zhang *et al.,* 2017), and nitric oxide (Cintra *et al.,* 2016) levels in saliva were measured by means of enzyme-linked immunosorbent assay kits (Glory science company, Ltd, USA and Invitrogen, California).

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS version 21 software of IBM. The data represented as mean± standard deviation. One way analysis of variance (ANOVA) was used for analysis of data. Bonferroni's test was used for post-hoc analysis for inter group comparisons. Differences between groups were considered significant at $p \leq 0.05$

RESULTS

A significant reduction was observed in pocket depth and clinical attachment level ($p \leq 0.001$) in patients treated with

Table 1a: Measurement of oral health parameters before and after treatment (n=10)

Groups	Probing pocket depth		Clinical attachment level		Tooth mobility	
	Before treatment (n=10)	After treatment (n=10)	Before treatment (n=10)	After treatment (n=10)	Before treatment (n=10)	After treatment (n=10)
Standard treatment group	3.89 ± 0.81	3.74 ± 0.93	3.75 ± 0.54	3.55 ± 1.00	0.60 ± 0.67	0.60 ± 0.67
Gel treatment group	3.27 ± 0.36	1.37 ± 0.25***	3.40 ± 0.52	1.69 ± 0.51***	1.10 ± 0.32	0.50 ± 0.53
Mouthwash treatment group	3.32 ± 0.14	1.45 ± 0.18***	3.41 ± 0.21	1.56 ± 0.18***	1.10 ± 0.32	0.50 ± 0.52

Table 1b: Measurement of oral health parameters before and after treatment (n=10)

Groups	Bleeding on probing		Plaque index		Gingival index	
	Before treatment (n=10)	After treatment (n=10)	Before treatment (n=10)	After treatment (n=10)	Before treatment (n=10)	After treatment (n=10)
Standard treatment group	0.89 ± 0.31	0.73 ± 0.24	2.62 ± 0.16	2.30 ± 0.14	2.62 ± 0.36	1.88 ± 0.76
Gel treatment group	0.85 ± 0.29	0.00 ± 0.00***	2.47 ± 0.42	0.33 ± 0.49***	2.48 ± 0.27	0.32 ± 0.22***
Mouthwash treatment group	0.87 ± 0.29	0.00 ± 0.00***	2.28 ± 0.64	0.35 ± 0.54***	2.31 ± 0.52	0.30 ± 0.32***

Mean ± SD. ***$p \leq 0.001$, shows significance with standard treatment

gel and mouthwash (table 1a). Gingival index, plaque index was also reduced with decreased bleeding in patients treated with gel and mouthwash ($p \leq 0.001$) (table 1b).

Table 2a, 2b and 2c shows PGE_2, TNF-α, and nitric oxide levels, which reduced in both treatment groups ($p \leq 0.001$) in comparison to conventional treatment group. Inflammatory biomarkers were reduced more in patients treated with simvastatin gel ($p \leq 0.001$) in comparison to group of patients treated with simvastatin mouthwash.

DISCUSSION

Inflammation of periodontal tissues is associated with the release of certain mediators such as tumor necrosis factor-α, interleukin 1β, interleukin 6, interleukin 8, matrix metalloproteinases and PGE_2 as well due to release of reactive oxygen species by neutrophils in increase amounts (Yucel-Lindberg and, Bage, 2013). As a consequence of disturbance in bone homeostasis; there is an increase in the osteoclastic activity and a decrease in osteoblastic activity which cause alveolar bone loss and degradation of connective tissue. This inflammation is due to the presence of increase number of anaerobic bacteria present in the dental plaque which releases proteases and lipopolysaccharide (Hienz *et al.,* 2015). Clinical signs and symptoms associated with periodontal diseases include edema, erythema, enlarged gingival tissues and bleeding. Certain secondary factors are also associated with increased inflammation of periodontal tissues such as pregnancy in which there is a hormonal imbalance in the body (Corbella *et al.,* 2016), some type of drugs (Antoniazzi *et al.,* 2016) and certain systemic diseases (Bui *et al.,* 2019). Environmental factors, including stress and smoking are also linked with periodontal diseases (Bawankar *et al.,* 2018). Disturbances in chemotaxis and migration of neutrophils, the process of phagocytosis and the release of mediators of inflammation occur in patients who are smokers, so these patients do not respond to the normal conventional periodontal treatment (Tymkiw *et al.,* 2011).

Various traditional drugs are used to modulate these inflammatory responses. Anti-inflammatory drugs have an important role in inhibition of prostaglandin, pro-inflammatory cytokines and matrix metalloproteinases, which decreases the severity of the disease by reducing inflammation (Reddy *et al.,* 2003). Systemic antibiotics are also used adjunct to periodontal treatment to treat infection (Ong *et al.,* 2019). However, high doses of both these agents are essential to attain therapeutic concentrations at target sites which lead to increased risks of developing side effects such as GI bleeding and allergic reactions (Pretzl *et al.,* 2019). Since last 30 years, anti-inflammatory agents and antibiotics by the local drug delivery system have been used to treat periodontal diseases (Szulc *et al.,* 2018). Local drug delivery provides adequate concentration of drug at target sites without any systemic side effects (Jepsen and Jepsen, 2016).

Researchers found that statins have additional anti-inflammatory, anti-bacterial, immune-modulatory, anti-thrombotic and endothelium stabilizing effects with its lipid lowering effects. It is found to be effective in the inhibition of pro-inflammatory cytokines, including

Table 2a: Levels of PGE_2 (pg/ml) before and after treatment

Groups	Before treatment (n=10)	After treatment (n=10)
Standard treatment	94.60 ± 2.12	90.23 ± 2.45
Gel treatment group	92.09 ± 1.13	22.78 ± 1.53$^{***^{\wedge\wedge\wedge}}$
Mouthwash treatment group	97.83 ± 2.39	39.13 ± 2.64***

Table 2b: Levels of TNF-α (pg/ml) before and after treatment

Groups	Before treatment (n=10)	After treatment (n=10)
Standard treatment	71.45 ± 1.82	69.49 ± 1.23
Gel treatment group	79.08 ± 2.28	10.33 ± 2.13$^{***\wedge\wedge\wedge}$
Mouthwash treatment group	78.28 ± 1.58	17.52 ± 1.66***

Table 2c: Levels of Nitric oxide (µmol/ml) before and after treatment

Groups	Before treatment (n=10)	After treatment (n=10)
Standard treatment	61.44 ± 0.71	60.12 ± 0.52
Gel treatment group	66.29 ± 2.24	19.8 ± 2.48$^{***\wedge\wedge\wedge}$
Mouthwash treatment group	64.94 ± 2.32	30.73 ± 2.64***

Mean±SD. ***p≤ 0.001, shows significance with standard treatment.$^{\wedge\wedge\wedge}$ p≤ 0.001, shows the significance between gel and mouthwash

interleukins, TNF-α, IFN-γ from different cells, thus offers immunomodulatory effects. These effects seem beneficial in the treatment of gingivitis and periodontitis (Petit *et al.,* 2019). In this study our prepared gel of simvastatin reduced inflammation in patients with gingivitis and periodontitis, thus reducing the probing pocket depth, improves attachment level, and decrease bleeding (p≤0.001). Gingival and plaque index were also reduced (p≤0.001). The mouthwash preparation of 1% simvastatin also shows great results with the reduction in all parameters observed before and after treatment. However, a change in the degree of tooth mobility was not observed by both gel and mouthwash (p≥0.05). This may be due to the limitation of our study duration as many investigators (Agarwal *et al.,* 2016; Pradeep and Thorat, 2010) in their studies proved bone gain after six months by the use of simvastatin gel by placing it in periodontal pockets of patients with periodontal diseases.

This study noted great reduction in the levels of PGE_2, TNF-α, and nitric oxide in patients received treatment with gel (group B) and mouthwash (group C) after scaling and root planning in comparison to the patients undergoing only scaling and root planning (group A). This property of simvastatin in reducing the inflammatory mediators proved it as a potent anti-inflammatory, immunomodulatory and endothelial stabilization affecting agent (Petit *et al.,* 2019).

This study also found that inflammatory biomarkers were highly reduced in patients received gel of simvastatin as compared to the group received mouthwash. This may be due to the viscosity of gel and its mucoadhesiveness which makes it an ideal choice in treating gingivitis and periodontitis. Due to these properties the gel remains in periodontal pocket for a long period without affecting its efficacy (Aslani *et al.,* 2016). Mouthwash is also effective in reducing the inflammation of gingiva and periodontium and the inflammatory biomarkers to a significant level which makes it a good option to be used via local drug delivery system in providing immediate relief from inflammation, pain and bleeding.

CONCLUSION

Our study revealed that 1% simvastatin gel and mouthwash showed favorable results clinically by reducing periodontal parameters and inflammatory biomarkers. Thus we strongly suggest the use of simvastatin by local drug delivery system as an adjunct treatment of scaling and root planning. Besides the limitation of this study which includes small population and short time period, the results are promising and encouraging that is why more studies should be done on larger scale to acquire its full benefits in the treatment of periodontal diseases.

REFERENCES

Agarwal S, Chaubey KK, Chaubey A, Agarwal V., Madan E and Agarwal MC (2016). Clinical efficacy of subgingivally delivered simvastatin gel in chronic periodontitis patients. *J. Indian Soc Periodontol*., **20**(4): 409-416.

Antoniazzi RP, Zanatta FB, Rosing CK and Feldens CA (2016). Association among periodontitis and the use of crack cocaine and other illicit drugs. *J Periodontol*., **87**(12): 1396-1405.

Aslani A and Malekpour N (2016). Design, formulation, and physicochemical evaluation of periodontal propolis mucoadhesive gel. *Dent Res. J. (Isfahan)*, **13**(6): 484-493.

Bawankar PV, Kolte AP and Kolte RA (2018). Evaluation of stress, serum and salivary cortisol and interleukin-1β levels in smokers and non-smokers with chronic periodontitis. *J. Periodontol*., **89**(9): 1061-1068.

Bui FQ, Almeida-da-Silva CLC, Huynh B, Trinh A, Liu J, Woodward J, Asadi H and Ojcius DM (2019). Association between periodontal pathogens and systemic disease. *Biomed. J*., **42**(1): 27-35.

Caygur A, Albaba MR, Berberoglu A and Yilmaz HG (2017). Efficacy of glycine powder air-polishing combined with scaling and root planning in the treatment of periodontitis and halitosis: A randomised clinical study. *J. Int. Med. Res*., **45**(3): 1168-1174.

Cintra LT, Samuel RO, Azuma MM, de Queiroz AO, Ervolino E, Sumida DH, de Lima VM and Gomes-Filho JE (2016). Multiple apical periodontitis influences serum levels of cytokines and nitric Oxide. *J. Endod*., **42**(5): 747-51.

Corbella S, Taschieri, S, Del Fabbro M, Francetti L, Weinstein R and Ferrazzi E (2016). Adverse pregnancy outcomes and periodontitis: A systematic review and meta-analysis exploring potential association. *Quintessence Int.,* **47**(3): 193-204.

Cunha-Cruz J, Saver B, Maupome G and Hujoel PP (2006). Statin use and tooth loss in chronic periodontitis patients.*J.Periodontol*., **77**(6): 1061-1066.

Emani S, Gunjiganur GV and Mehta DS (2018). Determination of the antibacterial activity of simvastatin against periodontal pathogens, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An *in vitro* study. *Contemp Clin Dent*., **5**(3): 377-82.

Gunjiganur Vemanaradhya G, Emani S, Mehta DS and Bhandari S (2017). Effect of 1.2% of simvastatin gel as a local drug delivery system on gingival crevicular fluid interleukin-6 & interleukin-8 levels in non surgical treatment of chronic periodontitis patients. *Arch Oral Biol*., **82**: 55-61.

Gupta A, Govila V and Saini A (2015). Proteomics - The research frontier in periodontics. *J. Oral. Biol. Craniofac. Res*., **5**(1): 46-52.

Hasturk H, Kantarci A and Van Dyke TE (2012). Paradigm shift in the pharmacological management of periodontal diseases. *Front Oral Biol*., **15**: 160-76.

Hienz SA, Paliwal S and Ivanovski S (2015). Mechanisms of bone resorption in periodontitis. *J. Immunol. Res*., **2015**: 615486.

Jepsen K and Jepsen S (2016). Antibiotics/antimicrobials: Systemic and local administration in the therapy of mild to moderately advanced periodontitis. *Periodontol.,* **71**(1): 82-112.

Kaminska M, Aliko A, Hellvard A, Bielecka E, Binder V, Marczyk A, Potempa J, Delaleu N, Kantyka T and Mydel P (2018). Effects of statins on multispecies oral biofilm identify simvastatin as a drug candidate targeting *Porphyromonas gingivalis*. *J. Periodontol.,* **90**(6): 637-646.

Kim A, Kim JH, Kim A, Sohn Y, Cha JH, Bak EJ and Yoo YJ (2018). Simvastatin attenuates tibial bone loss in rats with type 1 diabetes and periodontitis. *J. Transl. Med*., **16**(1): 306.

Ong A, Kim J, Loo S, Quaranta A and Rincon AJC (2019). Prescribing trends of systemic antibiotics by periodontists in Australia. *J. Periodontol*., doi: 10.1002/JPER.18-0586.

Pei X Y, He L and Ouyang XY (2017). Proposing a novel, three-level definition of periodontitis using probing depth, clinical attachment loss and bleeding on probing: analysis of a rural chinese population. *Chin. J. Dent. Res*., **20**(2): 89-96.

Petit C, Batool F, Bugueno IM, Schwinte P, Benkirane-Jessel N and Huck O (2019). Contribution of statins towards periodontal treatment: A Review. *Mediators Inflamm*., **2019**: 6367402.

Pradeep AR and Thorat MS (2010). Clinical effect of subgingivally delivered simvastatin in the treatment of patients with chronic periodontitis: A randomized clinical trial. *J. Periodontol*., **81**(2): 214-22.

Prasad S, Tyagi AK and Aggarwal BB (2016). Detection of inflammatory biomarkers in saliva and urine: Potential in diagnosis, prevention, and treatment for chronic diseases. *Exp. Biol. Med.*, **241**(8): 783-99.

Pretzl B, Salzer SB, Schlagenhauf U, Dannewitz B, Dommisch H, Eickholz P and Jockel-Schneider Y (2019). Administration of systemic antibiotics during non-surgical periodontal therapy-a consensus report. *Clin. Oral Investig*, **23**(7): 3073-3085.

Ranjan R, Patil SR and HRV (2017). Effect of in-situ application of simvastatin gel in surgical management of osseous defects in chronic periodontitis-A randomized clinical trial. *J. Oral Biol. Craniofac Res*., **7**(2): 113-118.

Reddy MS, Geurs NC and Gunsolley JC (2003). Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents. A systematic review. *Ann. Periodontol*., **8**(1): 12-37.

Szulc M, Zakrzewska A and Zborowski J (2018). Local drug delivery in periodontitis treatment: A review of contemporary literature. *Dent. Med. Probl*., **55**(3): 333-342.

Tymkiw KD, Thunell DH, Johnson GK, Joly S, Burnell KK, Cavanaugh JE, Brogden KA, Guthmiller JM (2011). Influence of smoking on gingival crevicular fluid cytokines in severe chronic periodontitis. *J. Clin. Periodontol*., **38**(3): 219-228.

Yucel-Lindberg T and Bage T (2013). Inflammatory mediators in the pathogenesis of periodontitis. *Expert Rev Mol Med*., **1 5**: e7.

Zhang J, Zhang AM, Zhang ZM, Jia JL, Sui XX, Yu LR and Liu HT (2017). Efficacy of combined orthodontic-periodontic treatment for patients with periodontitis and its effect on inflammatory cytokines: A comparative study. *Am. J. Orthod. Dentofacial. Orthop*., **152**(4): 494-500.