doi.org/10.36721/PJPS.2020.33.5.REG.2053-2058.1

Effectiveness of local drug delivery system using 1% metronidazole gel and mouthwash in treating periodontal diseases

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Abstract: The gold standard therapy for treating the periodontal infections is the removal of bacterial plaque and deposits of calculus from tooth surfaces by scaling and root planning. In order to eliminate these bacterial reservoirs, beside conventional treatment, chemo therapeutic agents are commonly prescribed by periodontologists. To avoid the systemic side effects and development of antibiotics resistance, local drug delivery methods has gained the attention of dentists to treat periodontal infections, along with scaling and root planning. The aim of this study was to evaluate the effectiveness of local drug delivery system in combination with scaling and root planning, by using 1% metronidazole gel and mouthwash. The patients were divided into 3 groups. Group I: conventional treatment group. Group II: patients received treatment with gel. Group III: patients received treatment with mouthwash. All groups received treatment for 30 days. Clinical parameters and salivary concentration of TNF-α, PGE_2 and nitric oxide were measured before and after treatment in both groups. All clinical parameters and inflammatory biomarkers significantly reduced in gel and mouthwash group patients ($p \leq 0.001$) as compared to patients received conventional treatment. The gel is found to be more efficacious than mouthwash especially in reducing clinical attachment loss ($p \leq 0.05$) and in reducing inflammatory biomarkers ($p \leq 0.001$). We strongly suggest the use of metronidazole via local drug delivery system combined with scaling and root planning to treat periodontal diseases.

Keywords: Metronidazole, local drug delivery, scaling and root planning, inflammatory salivary biomarkers.

INTRODUCTION

The gold standard therapy for treating the periodontal infections is the removal of bacterial plaque and deposits of calculus from tooth surfaces by scaling and root planning. It shifts the bacterial biofilm to less infectious composition, thus modulating the host response (Nastri *et al.,* 2019). The complex nature of biofilm and many factors including the root concavities, furcation involvement, and anatomical irregularities of teeth make difficult to target anaerobic bacteria. These pathogens move towards cementum in lacunar defects and finally effects radicular dentine. These areas harbor the bacteria and they again recolonize at the treated surfaces of tooth and form a biofilm of more complex nature. In order to eliminate these bacterial reservoirs, in addition to scaling and root planning, chemo therapeutic agents are commonly prescribed by periodontologists which deliver additional advantage to conventional treatment (Pandit *et al.,* 2013).

Antibiotics are mostly prescribed by the dentists initially at the phase of diagnosis of periodontal diseases (Feres *et al.,* 2018). Due to the broad spectrum antimicrobial activity of metronidazole, it is still used as a drug of choice by clinicians in treating periodontal infections. Metronidazole is one of the most common drug used in combination with amoxicillin (Ong *et al.,* 2019). Large doses of metronidazole are required to produce the desired effective therapeutic concentration at the target diseased sites which leads to the development of several side effects such as nausea, vomiting, headache, hypersensitivity and GI bleeding (Zandbergen *et al.,* 2016).

To avoid the systemic side effects and development of resistance associated with the use of antibiotics, local drug delivery methods has gained the attention of dentists to treat periodontal infections, in combination with scaling and root planning. Local drug delivery system was introduced by Goodson *et al*. in 1976. So from the last 40 years researches are going on different formulations of antibiotics and anti-inflammatory agents to use it via local drug delivery system to treat periodontal diseases and researchers found variable results in this regard (Rocha *et al.,* 2015).

This system provides high concentration of antibiotic in the periodontal pockets only at the affected area and thus treats the infection without side effects and less chances of resistance development (Bayramov and Neff, 2017). Different chemotherapeutic agents are available which are delivered locally to treat the periodontal diseases such as in the form of chips (Lewis *et al.,* 2011), fibers (Luo *et al.,* 2016), gels (Hoque *et al.,* 2018) and oral rinses (Costa *et al.,* 2017).

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Several studies are done on different drug delivery systems of metronidazole used in periodontal diseases and the researchers found positive results when the drug is applied locally rather systemically (Paul *et al.,* 2015; Re *et al.,* 2016; Nastri *et al.,* 2019).

In this study we tried to evaluate the effectiveness of local drug delivery system in combination with scaling and root planning, by using 1% metronidazole gel and mouthwash.

MATERIALS AND METHODS

Subject population

The study was conducted as a parallel group randomized clinical control trial. The patients were selected in-between February, 2018 to July, 2018. The patients who were agreed to register in the study signed the consent form. The study was conducted in the laboratory of Pharmacology Department of Karachi University, and HEJ Research Institute of Chemistry Karachi University. The study was done after approval from board of advanced studies and research (BASR) KU and the Independent Ethics Committee of International Center for Chemical and Biological sciences ICCBS/IEC-029-HS2017/Protocol/1.0.

Medical and dental history of the agreed participants was taken before initiating the clinical procedures. All methods in this study involving human subjects were according to the Helsinki declaration, 2013.

Included patients are those who were diagnosed with gingivitis and periodontitis without any systemic diseases and more than 18 years of age. Patient's exclusion criteria were pregnant females and lactating mothers, patients which are on medicines from six months and patients who got any periodontal treatment since last two months

The study was divided in to three phases

Pharmacological phase

In this phase 500gm gel was prepared by adding 5 gm of metronidazole in propylene glycol (Sallam *et al.,* 2015) on magnetic hot plate with stirrer (IKA Works Inc.). When a clear solution was obtained it was added in 1% carbapol 940 gel with preservatives (Razzaq *et al.,* 2018). For adjusting the pH of gel triethanolamine was added in the preparation. The gel was assessed for its pH, syringeability, spreadability, mucoadhesiveness and viscosity after preparation. Minimum inhibitory concentration of gel was measured by using agar diffusion method. Finally total microbial count of the preparation was done for quality assurance (Swain *et al.,* 2019).

1000 ml of 1% metronidazole mouthwash was prepared by adding 10 gm metronidazole in benzyl alcohol. Preservative and triethanolamine were added to avoid the fungal growth and adjust the mouthwash's pH respectively. Food color and flavor then finally added so that it could be tolerated by patients. Mouthwash was assessed for its pH (Anshula *et al.,* 2018). Minimum inhibitory concentration of mouthwash was measured by using agar diffusion method (Joy *et al.,* 2017). Total microbial count of the preparation was done for quality assurance (Ardakani *et al*., 2015).

Clinical phase

In the clinical phase trial 30 patients (15 male and 15 female) were selected and included in the study. The patients were randomly divided into three groups. Group I: treated with scaling and root planning only for a period of 30 days; Group II: treated with 1% gel preparation after scaling and root planning for a period of 30 days; Group III: treated with 1% mouthwash preparation after scaling and root planning for a period of 30 days.

The gel treatment group received 1% metronidazole gel with a flexible, blunt 25 gauge needle, deep into the periodontal pockets without causing harm and damage to periodontal tissues.

Six clinical variables were estimated at baseline before initiating the treatment and then after four weeks of treatment. The following parameters were estimated: probing pocket depth, attachment level (Jeyasree *et al.,* 2018), plaque index, gingival index (Ashouri *et al.,* 2017), tooth mobility and bleeding on probing (Zhang *et al.,* 2017).

Collection of saliva samples and laboratory estimation of inflammatory biomarkers

Normal standard protocol was followed for collection of saliva. The patients were instructed to wash their mouth with water in order to clean out exfoliated cells. 3 to 5 ml of unstimulated saliva was collected in the morning between 9am to 11am. After collecting the samples in sterile vials it was centrifuged instantly (Jeyasree *et al*., 2018). After centrifugation, the obtained clear fluid was assessed for TNF-α (Moura *et al*., 2017) {INVITROGEN, California, USA}, PGE_2 (Gumuş *et al*., 2017) {GLORY SCIENCE CO., LTD, USA} and nitric oxide (Ozdemir *et al.,* 2016) {GLORY SCIENCE CO., LTD, USA} levels by using ELISA kits.

STATISTICAL ANALYSIS

Computer software SPSS version 21of IBM was used for analysis of data. Mean values with standard deviations were used for the calculation of clinical variables and biochemical variables in all groups before initiating the treatment and after treatment completion. One way ANOVA was used for data analysis and for inter-group comparison Bonferroni's test was used. $p \leq 0.05$ was considered significant.

RESULTS

Pocket depth, clinical attachment level, mobility of tooth, bleeding, plaque and gingival index were highly reduced by gel of metronidazole ($p \leq 0.001$) as compared to conventional treatment. The gel reduces the clinical attachment loss slightly more as compared to mouthwash ($p \leq 0.05$) (table1 & 2).

Mouthwash preparation reduces pocket depth, mobility of tooth, bleeding, plaque and gingival index to highly significant level ($p \leq 0.001$) as compared to conventional treatment. Clinical attachment level was also reduced to a significant level ($p \leq 0.01$) as compared to conventional treatment (table1 & 2).

Concentration of inflammatory biomarkers were reduced by both gel and mouthwash preparations ($p \leq 0.001$) as compared to conventional treatment. The gel found to be more effective than mouthwash in reducing inflammatory biomarkers ($p \leq 0.001$) (table 3, 4, &5).

DISCUSSION

Prolonged and unselective utilization of systemic antibiotics results in the development of resistant microbial strains and the chances of causing untoward systemic adverse effects. Therefore, local drug delivery system is found to be an effective medium to treat periodontal diseases. Delivered drug can stay in the periodontal pockets for a longer period of time to produce the desired effects without disrupting the microbial balance in the oral cavity and development of resistant microbial strains and devoid of causing systemic adverse effects (Bottino *et al*., 2014; Liang *et al*., 2020).

Assem *et al*. in 2017 and Suryaprasanna *et al*. in 2018 in their studies observed reduction in clinical parameters and decrease in periodontopathic organisms after administering systemic antibiotic along with scaling and root planning during periodontal therapy. Here we found that only local application of 1% metronidazole gel and mouthwash after scaling and root planning, there is a significant reduction in all clinical parameters in a very short period of four weeks, without reporting any side effects.

In a previous study carried out in 2018, Feres and colleagues found that only scaling and root planning fails to remove the anaerobic pathogens completely from the subgingival tissue and may increase clinical attachment loss that results in increase tooth mobility. We found that the patients received gel application after scaling and root planning had improved clinical attachment levels in comparison to the group of patients received mouthwash treatment after scaling and root planning. This is due to the mucoadhesive ability of the gel to retain for longer time period in the subgingival pocket with slow drug release. This finding was in agreement with the previous studies done by Nastri *et al*. in 2019. Improved clinical attachment level marks in reduced gingival pocket depth which ultimately results in decreased tooth mobility.

Bleeding on probing, plaque index and gingival index were also improved significantly in both treatment groups received gel and mouthwash preparations due to changes in wall of periodontium brought about by resolution of inflammation. A similar finding was also reported by Kurgan *et al*. in 2018.

In a recent study, Martin *et al*. in 2019 evaluated clinical parameters and immune responses in patients with aggressive periodontitis after conventional scaling and root planning along with systemic antibiotics. His study showed improvement in clinical parameters and reduction in inflammatory biomarkers including TNF-α. Our study found better results than Martin *et al*. (2019) in which we proved that local use of only 1% metronidazole gel and mouthwash reduced the TNF-α to highly significant level.

Taiete *et al*. in 2016 reported that PGE_2 levels were highly reduced in patients of periodontitis received non-surgical periodontal therapy in combination with oral metronidazole but in our study PGE_2 levels were dropped down with the use of only 1% gel and mouthwash. The levels of nitric oxide, another important mediator of inflammation were also found to be decreased with both 1% gel and mouthwash preparations. The gel preparation is found to be more effective than mouthwash in reducing all inflammatory biomarkers, due to its retentive and mucoadhesive properties.

CONCLUSION

There are several advantages of treating patients of gingivitis and periodontitis with antibiotics via local drug delivery system. The most significant advantage is that this system provides 100 times higher concentration of antibiotic in subgingival periodontal pockets as compared to the dose which is given systemically in normal dosage. This system also permits broad margin for other antibiotics and drugs to be used in treating gingivitis and periodontitis, which are not used systemically in treating these conditions. Other benefits includes reduction in problems of patients compliance, decreases the chances of drug resistance development and side effects of drug linked with its oral use.

Thus, we strongly suggest the use of metronidazole via local drug delivery system combined with scaling and root planning to treat periodontal diseases and controlled use of systemic antibiotics is the best approach to elude the adverse effects and decrease its worldwide resistance.

Table 1: Periodontal parameters at base line and after four weeks of treatment

Groups	Pocket depth		Clinical attachment level		Tooth mobility	
	Baseline (n=10)	After four weeks (n=10)	Baseline (n=10)	After four weeks (n=10)	Baseline (n=10)	After four weeks (n=10)
Conventional treatment group	4.04 ± 0.81	4.00 ± 0.98	4.25 ± 0.54	4.15 ± 1.00	0.70 ± 0.67	0.70 ± 0.67
Gel treatment group	4.37 ± 1.23	1.84 ± 1.16***	4.56 ± 1.16	2.48±0.83***¥	0.60 ± 0.52	0.40 ± 0.52***
Mouthwash treatment group	4.08 ± 0.69	2.00 ± 1.02***	4.30 ± 0.70	3.34 ± 1.01**	0.70 ± 0.82	0.20 ± 0.42***

Values are plotted as mean± standard deviation. ** p≤0.01, *** p≤0.001 is considered significant with conventional treatment. ¥ p≤0.05 shows the significance between gel and mouthwash.

Table 2: Periodontal parameters at base line and after four weeks of treatment

Groups	Bleeding on probing		Plaque index		Gingival index	
	Baseline (n=10)	After four weeks (n=10)	Baseline (n=10)	After four weeks (n=10)	Baseline (n=10)	After four weeks (n=10)
Conventional treatment group	0.90 ± 0.32	0.54 ± 0.25	2.64 ± 0.18	2.31 ± 0.15	2.63 ± 0.37	1.89 ± 0.77
Gel treatment group	1.00 ± 0.00	0.13 ± 0.23***	2.58 ± 0.45	0.68 ± 0.51***	2.52 ± 0.36	0.74 ± 0.65***
Mouthwash treatment group	0.93 ± 0.21	0.02 ± 0.05***	2.52 ± 0.18	0.42 ± 0.51***	2.41 ± 0.17	0.55 ± 0.71***

Values are plotted as mean± standard deviation. *** p≤0.001 is considered significant with conventional treatment.

Table 3: Concentration of TNF-α (pg/ml) in saliva at base line and after four weeks of treatment

Groups (n=10)	Before treatment	After treatment
Conventional treatment group	74.26 ± 1.78	65.67 ± 1.58
Gel treatment group	78.03 ± 1.23	3.15 ± 1.19***¥¥¥
Mouthwash treatment group	71.05 ± 2.25	19.91 ± 2.31***

Table 4: Concentration of PGE_2 (pg/ml) in saliva at base line and after four weeks of treatment

Groups (n=10)	Before treatment	After treatment
Conventional treatment group	94.90 ± 2.26	92.9 ± 2.47
Gel treatment group	84.57 ± 1.23	7.28 ± 1.56*** ¥¥¥
Mouthwash treatment group	86.52 ± 0.91	16.86 ± 1.21***

Table 5: Concentration of Nitric oxide (µmol/ml) in saliva at base line and after four weeks of treatment

Groups (n=10)	Before treatment	After treatment
Conventional treatment group	62.47 ± 0.75	61.72 ± 0.67
Gel treatment group	65.13 ± 2.29	29.35 ± 2.83***¥¥¥
Mouthwash treatment group	60.27 ± 0.76	45.03 ± 0.48***

Values are plotted as mean± standard deviation. *** p≤0.001 is considered significant with conventional treatment. ¥¥¥ p≤0.001 the significance between gel and mouthwash.

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