Original Article

Effects of a single episode of subgingival irrigation with tetracycline HCl or chlorhexidine: A clinical and microbiological study

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Abstract:

Background: The present study was designed to evaluate clinical and antimicrobial effects of a single episode of subgingival tetracycline or chlorhexidine (CHX) irrigation in the absence of scaling and root planing. **Materials and Methods:** Thirty patients diagnosed with chronic periodontitis were recruited for this study, each providing four non-adjacent untreated periodontal pockets with a probing depth equal to or exceeding 6 mm. The four deep periodontal pockets in each patient were assigned to be irrigated with 150 ml each of CHX digluconate 0.2% (group A), tetracycline HCl at concentrations of 10 and 50 mg/ml (groups B and C, respectively), or sterile saline (group D) in a single episode. Recordings of plaque index (PI), gingival index (GI), probing pocket depth (PD), clinical attachment level (CAL), and subgingival bacterial counts from paper point samples were made pre-irrigation (day 0), and at days 7, 28, 56, and 84, post-irrigation. **Results:** The mean PI and GI scores for all the four groups were reduced post-irrigation, the reduction being significantly higher in group C compared to the other groups. The reduction in the PD and CAL was more significant in group C from day 0 to day 7, whereas the other groups showed minimal changes during this period. The change in the bacterial count was altered towards one of the periodontal health, it being more significant and consistent in the group C. **Conclusion:** The results of this study suggest that subgingival irrigation with high concentrations of tetracycline may play a beneficial role in the management of chronic periodontitis patients.

Key words:

Chlorhexidine, cocci, spirochetes, subgingival irrigation, tetracycline

Access this article online

Website: www.jisponline.com

DOI: 10.4103/0972-124X.85668

Quick Response Code:

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Submission: 18-08-2010
Accepted: 09-05-2011

INTRODUCTION

The periodontal diseases represent a group of localized microbial-induced infections involving the gingiva and supporting tissues of the teeth. There is considerable evidence implicating facultative and obligate anaerobic bacteria as the primary cause of periodontal disease.[1] The control of the prevalence and severity of periodontal disease implies a managed reduction of either the total microbial plaque mass or at least those microbes considered to be pathogenic. This is performed routinely in the dentist's office by mechanical scaling and root planing (SRP). However, variation in the ability of the therapist to gain access to deep and tortuous pockets often results in substantial variation in the effectiveness of SRP. This has led to the adjunctive use of antibacterial agents, usually in the form of irrigants or systemic antibiotics. In the treatment of periodontal diseases, irrigation is used as a lavage to flush away the bacteria that are in contact with the periodontal tissues. Oral irrigation has been promoted and used as a dental hygiene aid for over 80 years. Recent evidence has suggested that oral irrigation is effective in altering, both quantitatively and qualitatively, the unattached subgingival plaque associated with chronic adult periodontitis.[2]

Chlorhexidine (CHX), a bisbiguanide compound, has been shown to possess a broad spectrum of topical antimicrobial activity. It is this property, in addition to the safety, effectiveness, substantivity, lack of serious side effects and lack of toxicity that has allowed it to be used extensively in dentistry, usually as a mouthrinse. The combined use of irrigators and CHX appears to be more effective than when used as a mouthrinse at altering the subgingival microflora[3] The tetracyclines are a group of bacteriostatic antimicrobials effective against a wide range of organisms and successfully tested in both animal models and clinical studies. Preliminary data indicate that tetracycline HCl may be retained in the subgingival environment and is slowly released to the gingival crevicular fluid in clinically meaningful concentrations following an irrigation procedure. It is not known, however, whether this was an effect

of substantivity of tetracycline HCl to the periodontally exposed root or to other subgingival tissues.[4] The aim of this present study was to compare and evaluate the clinical and antimicrobial effects of a single episode of subgingival irrigation with 150 ml each of 0.2% CHX digluconate, tetracycline HCl at 10 mg/ml and 50 mg/ml concentrations and normal saline, in the absence of SRP.

MATERIALS AND METHODS

Thirty patients diagnosed with chronic periodontitis were selected.

Inclusion criteria

1. The patients belonged to age group, ranging from 25 to 65 years
2. Four sites were selected in each patient, exhibiting periodontal pockets with a probing depth of >6 mm
3. The selected sites were separated by at least two teeth

Exclusion criteria

1. Patients afflicted with serious, uncontrolled medical disorders
2. Patients who had undergone SRP procedures within the immediate past six months
3. Patients who had taken antibiotics within the past six months
4. Patients who are known to be sensitive to CHX or tetracycline

Study design

The selected sites were randomly assigned to four groups, each receiving one out of the four irrigants [Figure 1] and were designated as follows:
Group A: 0.2% CHX
Group B: Tetracycline at 10 mg/ml concentration (TTC 10)
Group C: Tetracycline at 50 mg/ml concentration (TTC 50)
Group D: Sterile saline

Within one month prior to study initiation, the patients received oral hygiene instructions, and underwent SRP procedures. Experimental teeth and immediately adjacent teeth did not receive instrumentation. On day 0, the clinical parameters were recorded at the selected sites and subgingival plaque samples were collected, prior to irrigation. The following sequence of recording and microbial sampling was used for the study sites:

1. Plaque index (Sillness and Loe, 1964)
2. Gingival index (Loe and Sillness, 1963)
3. Microbial sampling
4. Probing pocket depth
5. Clinical attachment level

Collection of subgingival plaque sample

Plaque samples were collected from all the four sites at baseline and subsequent recall visits of the patient. Each site was isolated with cotton rolls and the supragingival plaque was cleared with sterile cotton pellets. Three paper points were introduced successively into the depth of the periodontal pocket for 10 s each to harvest subgingival plaque [Figure 2]. After removal from the pocket, the apical 3 mm of each point was cut and immediately placed into a vial containing 3 ml of pre-reduced anaerobically sterilized Ringer's solution. Samples were dispersed by vortexing at maximal setting for 60s.

Microscopic examination

Within one hour of sampling, a drop of the dispersed plaque sample was mounted on a glass slide and examined under the phase contrast microscope for the presence and distribution of spirochetes, rods, and cocci. For each plaque sample the bacterial counts were made for two separate fields and subsequently their mean was calculated.

Preparation of tetracycline solutions

The number of capsules needed for obtaining the desired concentrations was calculated and are given below:
For TTC10: 3 (500 mg) tetracycline HCl capsules
For TTC50: 15 (500 mg) tetracycline HCl capsules

Tetracycline solutions were prepared by dissolving the contents of the calculated number of capsules into 150 ml double-distilled sterile water each. The mixture was stirred thoroughly at an approximate temperature of 53°C. Any remaining filler particles were filtered away.

Irrigation protocol

After isolation with cotton rolls, each selected site was subjected to subgingival irrigation of 150 ml of the allocated solution using subgingival tips of a mechanical irrigator emitting a pulsating jet of the solution. Excess irrigant solution was continuously aspirated [Figure 3]. The irrigation was carried out for an approximate period of 5 min for each selected site. Following the irrigation protocol, the patients were reinforced in their oral hygiene instructions. They were subsequently recalled at days 7, 28, 56, and 84 post-irrigation, and the clinical parameters were recorded at the selected sites and subgingival plaque samples were collected and examined as described previously.

Mean and standard deviation were estimated from the samples for each study group. Mean values were compared by Student's paired *t*-test. Student's *t*-test was used to calculate the *P*-value, and the reported *P*-values were adjusted for multiple comparisons by Bonferroni correction method. In the present study, $P<0.05$ was considered as the level of significance.

RESULTS

The mean plaque index and gingival index scores for all the four groups were reduced from day 0 to day 7 and maintained low levels through days 28, 56, and 84 [Tables 1 and 2]. The reduction in the mean plaque index score within group C was found to be significantly higher on day 7 as compared to other groups. Inter-group comparison showed that the mean plaque index scores for group C were more significantly reduced as compared to the other groups. The mean gingival index score for group C was significantly reduced on days 28, 56, and 84 as compared to the other groups. A reduction in the mean probing pocket depth values for all the four groups was observed [Table 3]. However, inter-group comparison showed that the reduction in the mean probing pocket depth for group C from day 0 to day 7 was significantly higher as compared to reduction in other groups. The mean clinical attachment level values showed minimal changes from day 0 to day 7 for all the groups, except for group C, which showed a



Figure 1: Solutions used for irrigation (from left to right): 2% chlorhexidine gluconate, TTC 10, TTC 50, sterile saline



Figure 2: Collection of subgingival plaque sample



Figure 3: Subgingival irrigation with tetracycline solution

statistically significant change [Table 4]. Significant gains were observed in groups B and C from day 28 onwards. The overall reduction in the mean probing pocket depth and gain in the mean clinical attachment level was higher for group C.

Table 1: Mean and standard deviation of Plaque Index scores at days 0, 7, 28, 56, and 84 for groups A, B, C, and D

Groups	Day 0	Day 7	Day 28	Day 56	Day 84
A (CHX)	1.9+0.8	1.5+0.6	1.4+0.5	1.3+0.5	1.4+0.5
B (TTC 10)	1.8+0.7	1.5+0.6	1.1+0.4	1.0+0.6	1.0+0.2
C (TTC 50)	1.9+0.6	1.2+0.6	0.8+0.5	0.6+0.5	0.7+0.5
D (SALINE)	2.0+0.7	1.6+0.6	1.5+0.5	1.2+0.5	1.3+0.5

CHX – Chlorhexidine

Table 2: Mean and standard deviation of Gingival Index scores at days 0, 7, 28, 56, and 84 for groups A, B, C, and D

Groups	Day 0	Day 7	Day 28	Day 56	Day 84
A (CHX)	2.7±0.5	1.9±0.4	1.8±0.5	1.6±0.5	1.5±0.5
B (TTC 10)	2.4±0.6	2.0±0.6	1.4±0.6	0.9±0.5	0.9±0.4
C (TTC 50)	2.6±0.5	1.4±0.6	0.8±0.6	0.6±0.6	0.5±0.5
D (SALINE)	2.7±0.5	2.0±0.7	1.8±0.5	1.4±0.5	1.5±0.5

CHX – Chlorhexidine

Table 3: Mean and standard deviation of probing pocket depth (mm) at days 0, 7, 28, 56, and 84 for groups A, B, C, and D

Groups	Day 0	Day 7	Day 28	Day 56	Day 84
A (CHX)	6.6±0.8	6.5±0.7	6.4±0.6	6.2±0.6	6.1±0.5
B (TTC 10)	7.0±0.7	6.9±0.7	6.4±0.5	6.0±0.6	5.9±0.5
C (TTC 50)	6.9±0.9	6.4±0.7	5.8±0.5	5.5±0.5	5.3±0.5
D (SALINE)	7.0±0.9	6.9±0.7	6.6±0.6	6.2±0.6	6.0±0.4

CHX – Chlorhexidine

Table 4: Mean and standard deviation of clinical attachment level (mm) at days 0, 7, 28, 56, and 84 for groups A, B, C, and D

Groups	Day 0	Day 7	Day 28	Day 56	Day 84
A (CHX)	7.0±0.1	7.1±1.1	6.8±0.9	6.7±0.7	6.6±0.6
B (TTC 10)	7.3±0.9	7.3±0.9	6.9±0.8	6.5±0.7	6.2±0.6
C (TTC 50)	7.3±0.9	6.9±0.8	6.2±0.6	5.7±0.6	5.6±0.7
D (SALINE)	7.2±1.0	7.1±0.9	6.9±0.8	6.6±0.7	6.6±0.6

CHX – Chlorhexidine

The mean spirochetes and motile rods' count showed statistically significant reduction post-irrigation for all groups except group A [Table 5]. The reduction in the spirochetes and motile rods' count was significantly higher in group C compared to the other groups on days 28, 56, and 84 and also remained consistent throughout the duration of the study. The mean cocci counts were increased post-irrigation in all groups, with the most significant changes seen in groups B and C [Table 6]. Inter-group comparison showed that the increase in the mean cocci scores were significantly higher in group C on days 28, 56, and 84.

DISCUSSION

Subgingival plaque removal and control is a fundamental objective of periodontal therapy. Conventional closed debridement of subgingival plaque from root surfaces can be effective, but is time consuming and technically demanding. It has been shown that the chance of failing to remove all of the subgingival plaque increases in pocket depths greater than 4 mm. As subgingival plaque accumulates and matures, periodontal breakdown can occur.[5] Therefore, professional maintenance therapy may not always achieve complete or

Table 5: Mean and standard deviation of spirochetes and motile rods' count at days 0, 7, 28, 56, and 84 for groups A, B, C, and D

Groups	Day 0	Day 7	Day 28	Day 56	Day 84
A (CHX)	5.2±3.6	4.1±3.3	3.9±2.5	3.4±2.1	4.4±2.2
B (TTC 10)	5.3±3.8	3.8±2.8	3.3±2.1	2.4±1.4	3.2±1.7
C (TTC 50)	5.3±3.2	3.1±2.8	1.8±1.6	1.2±1.2	1.5±1.5
D (SALINE)	4.7±3.8	3.8±3.3	3.2±2.4	2.6±1.7	4.0±1.8

CHX – Chlorhexidine

Table 6: Mean and standard deviation of cocci count at days 0, 7, 28, 56, and 84 for groups A, B, C, and D

Groups	Day 0	Day 7	Day 28	Day 56	Day 84
A (CHX)	2.2±1.3	2.4±1.3	2.3±1.4	2.7±1.6	2.5±1.2
B (TTC 10)	1.8±1.6	3.0±1.4	3.1±1.6	3.2±1.7	2.5±1.4
C (TTC 50)	2.3±1.5	3.6±1.5	4.1±1.5	4.1±1.4	3.9±1.0
D (SALINE)	2.2±1.4	2.9±1.4	2.7±1.5	2.7±1.7	2.4±1.5

CHX – Chlorhexidine

adequate debridement. This has led to the adjunctive use of antibacterial agents, usually in the form of irrigants or systemic antibiotics, to overcome the limited efficacy of the conventional treatment. The primary purpose of irrigation is to nonspecifically reduce the bacteria and their by-products that lead to the initiation or progression of periodontal diseases.

Local delivery of antibiotics and antimicrobials has been explored for the control of subgingival plaque. The potential benefits of an antimicrobial agent as an adjunct in periodontal therapy may be to disrupt, more intensely, the microflora or to alter, selectively, its regrowth so that mechanical therapy has a more prolonged effect.[6] The present study was designed to evaluate the clinical and antimicrobial effects of a single episode of subgingival irrigation of tetracycline or CHX in deep periodontal pockets.

In this study, the mean plaque index and gingival index scores for group C were more significantly reduced as compared to the other groups. Similar results were observed in a study done by Christersson *et al*.[7] to evaluate the binding to and release of tetracycline-HCl from human root dentin surfaces, *in vivo*. A significant improvement in the clinical parameters was seen during a three-month follow-up period. Silverstein *et al*.[8] found that tetracycline irrigation showed statistically significant improvement in clinical parameters when compared with control. The favorable results obtained in the above-mentioned studies may be due to the high concentration of tetracycline-HCl used (100 mg/ml) and repeated administration (every other day for a two-week period).

The main advantage of irrigation of periodontal pockets with tetracycline-HCl over systemic administration appears to be the localized concentration of the drug at the sites of disease activity, with minimal effects on the microflora present in other areas. The amount of drug delivered often creates sulcular medication concentrations exceeding the equivalent of 1 mg/ml. This level is considered bactericidal for the majority of bacteria that exhibit resistance to systemically delivered concentrations. Thus, the control of disease activity in deep periodontal pockets brought about by local delivery of the drug may be attributable to the improvement in the clinical parameters.[9]

The reduction in the mean probing pocket depth for group C from day 0 to day 7 was significantly higher as compared to reduction in other groups and only group C showed a statistically significant change in the mean clinical attachment level in the same time period. These results are similar to those observed by Stabolhz *et al*.[10] in a randomized, split-mouth clinical study, evaluating the results of a single episode of irrigation with CHX or tetracycline HCl. They suggested that subgingival irrigation with high concentrations of tetracycline solutions may have a role in the management of adult periodontitis. However, Trombelli *et al*.[11] showed that no adjunctive effect on the healing response was obtained by augmenting mechanical debridement with tetracycline. It is assumed that different levels of disease activity or disease severity were present at the time of treatment and could account for the lack of agreement between these studies.

The significant improvement in the clinical attachment levels of the sites irrigated with tetracycline HCl may be attributed to its' effects on periodontal regeneration. *In vitro* studies have shown enhanced soft tissue attachment to root surfaces treated with tetracycline. Dentin root surface demineralization by low pH tetracycline increases adsorption of fibronectin, an extracellular matrix glycoprotein. The adsorbed or bound fibronectin enhances fibroblast attachment and growth, while suppressing epithelial cell attachment and growth.[3]

The reductions in the spirochetes and motile rods' counts and increases in the mean cocci counts were significantly higher in group C compared to the other groups on days 28, 56, and 84. The changes in the subgingival bacterial counts in the present study concur with the results of the study done by Silverstein *et al*. Statistically significant improvements from baseline and from the control were found primarily for the tetracycline group. In this group, the cocci cell percentages were significantly elevated from both baseline and control values at two, four, and eight weeks associated with a statistically significant decrease in the percentage of spirochetes and motile rods.

Tetracycline acts by inhibiting protein synthesis in the bacterial cell. *in vitro* studies performed to assess antibiotic susceptibility showed that most potentially periodontopathic bacteria, including *P. intermedia, P. gingivalis, F. nucleatum* and *C. rectus*, are all susceptible to this antibiotic. The lipophilic properties of the tetracyclines explain their high penetration capacity into bacterial cells. Also, the crevicular fluid level of tetracycline was estimated to be two to four times the blood level at a given dose, far exceeding the minimum inhibitory concentrations of most periodontopathic bacteria.[1]

An important prerequisite for an antimicrobial drug as a plaque-inhibiting agent is substantivity, that is, the ability of the agent to adsorb to and subsequently desorb from surfaces such as those of teeth and oral soft tissues maintaining antimicrobial activity. Tetracycline is known to be a potent chelator and its' incorporation into enamel and dentin is caused by a yet unknown physiochemical process. Preliminary data indicate that tetracycline HCI may be retained in the subgingival environment to slowly release to the gingival crevicular fluid in clinically meaningful concentrations following an irrigation procedure. Stabholz *et al*.[12] demonstrated long-lasting

substantivity of tetracycline HCl to root dentin of extracted teeth, as compared to CHX digluconate. Roots immersed in tetracycline HCl at concentrations of 50 mg/ml and 10 mg/ml released antimicrobial activity for a significantly longer period (14 and 4 days, respectively). Interestingly, within each treatment, there were no differences between the exposure intervals (1, 3, or 5 min). The high substantivity of tetracycline HCl obtained in their study may be related to the high concentration (50 mg/ml) and quantity (150 ml for each site) used, which are similar to the present study. This demonstrates that the amount of antimicrobial activity retained is proportional to the concentration of tetracycline HCl used for irrigation.

Other studies have demonstrated multiple beneficial properties of tetracycline unrelated to its antimicrobial properties. Tetracycline HCI has been shown to etch and/or remove the root surface smear layer and cause surface demineralization, to delay pellicle and plaque formation, and to exhibit anti-collagenase activity as well as to inhibit, *in vitro*, parathyroid hormone-induced bone resorption and human neutrophil functions. It is apparent, therefore, that the effects of tetracycline may be multifactorial through modulation of both the subgingival microflora and mechanisms of tissue destruction.[11]

From this study it can be concluded that subgingival irrigation with tetracycline HCl at a concentration of 50 mg/ml has shown significant improvement in the clinical and microbial parameters compared to control. Further studies utilizing larger sample sizes and longer follow-up periods are recommended for supporting the findings of this study.

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How to cite this article: Krishna MK, Ravindran SK, Vivekanandan G, Navasivayam A, Thiagarajan R, Mohan R. Effects of a single episode of subgingival irrigation with tetracycline HCl or chlorhexidine: A clinical and microbiological study. J Indian Soc Periodontol 2011;15:245-9.

Source of Support: Nil, **Conflict of Interest:** None declared.