

Microbial Evaluation of a Single Subgingival Irrigation with Chlorhexidine and Benzylamine in Advanced Periodontitis

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ABSTRACT

Background: This study evaluated the microbial effects of a single subgingival irrigation of chlorhexidine and benzylamine in advanced periodontitis on a comparative basis in the absence of physical debridement using the microbiological parameter.

Methods: Patients who attended the Department of Periodontia, Madras Dental College were selected. The experimental site consisted of two teeth with Chronic Periodontitis, a pocket depth of 4 to 6 mm and a Gingival Index score of 2. The study has been designed to cover the following three groups:

- *Group I:* Irrigated with physiological saline (Control group).
- *Group II:* Irrigated with 0.2% chlorhexidine digluconate solution.
- *Group III:* Irrigated with 0.15% benzylamine hydrochloride solution.

A sample of 30 were selected, 10 samples in each group. The experimental sites of the three groups were assessed using the following Microbiological parameters: (1) spirochetes, (2) motile bacteria, (3) cocci, (4) other microorganisms.

Results: The microbiological analysis showed that the response of the Saline and Chlorhexidine with regard to the Spirochetes followed a concave pattern. But Benzylamine showed a slow tapering effect in annihilating the Spirochetes. In the case of Motile bacteria also the variation in the Saline was negligible and in the chlorhexidine group the trough occurred between first week and second week and then revived, whereas in Benzylamine alone there was no question of revival but the Motile bacteria followed the pattern of sine curve. With regard to Cocci though Saline showed good response it was not significant, whereas chlorhexidine and benzylamine recorded a highly appreciable response. In the case of other microorganisms Saline had no significant effect, whereas Chlorhexidine had significant effect at the end of 1 week and Benzylamine had a sea-saw effect throughout the different periods of time.

Conclusion: The subgingival action of Benzylamine can be favored and preferred to that of Chlorhexidine and Saline because of its sustained progressive response.

Keywords: Subgingival irrigation, Periodontitis, Chlorhexidine, Benzylamine.

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INTRODUCTION

Many documentary and paleopathological studies prove that periodontal diseases have been known to affect mankind since the beginning of recorded history.¹ The periodontal diseases represent a group of localized microbial-induced infections involving the gingiva and supporting tissues of the teeth. Knowledge concerning the role of microorganisms in the initiation of gingivitis and progression of periodontitis increased dramatically following the recognition of bacterial plaque as the major etiological factor (Loe et al 1965, Theilade et al 1966).²

Listgarten and Heliden have documented the relative proportion of subgingival morphotypes in periodontally healthy and diseased sites. Coccoid cells, straight rods, filaments and fusiforms predominate in healthy sites, while increased proportions of motile rods and spirochetes are found in diseased sites.³ Evidence shows that removal of subgingival plaque may not only lead to a reduction of probing depth but also to a change in the microflora,⁴ thus arresting the progression of the destructive periodontal disease.⁵ Various broad spectrum antibiotics have been tried and found to have an inhibitory effect on both caries and periodontitis. Recent approaches to the problem of subgingival plaque control include the use of chemical and antimicrobial agents. An antimicrobial agent used as a mouthwash does not affect periodontal pockets deeper than 3 mm. However insertion of the irrigation needle even 3 mm within the pocket resulted in penetration of an aqueous solution to the apical plaque border, regardless of pocket depth. This system has been shown to produce reductions in plaque and apparent periodontitis.⁶

Chlorhexidine has been found to inhibit the formation of plaque, prevent the development of gingivitis and cure experimentally induced gingival inflammation in humans.¹ Chlorhexidine incorporated in periodontal pack shows lesser

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plaque index and gingival scores.⁷ The local application of Chlorhexidine subgingivally may lead to changes in the subgingival flora which selectively interferes with the re-establishment of a pathogenic flora.

An addition to the therapeutic armamentarium is Benzylamine hydrochloride 0.15%, a nonsteroidal anti-inflammatory agent with antimicrobial properties.⁸ The increasing realization of bacterial specificity in destructive periodontal disease has focused upon the use of microbiological parameters to determine disease activity.⁹ In trials in which the efficacy of certain therapeutics have to be evaluated it is of interest to analyze changes of subgingival micro biota of one particular site which occur from one interval to another.¹⁰

The Present study has been conducted so far on the antimicrobial effects of a single subgingival irrigation of Chlorhexidine and Benzylamine on a comparative basis in the absence of physical debridement using bacterial parameters of periodontal disease.

MATERIALS AND METHODS

Patients who attended the Department of periodontia, Madras Dental College and clearly satisfying the following clinical criteria have been taken as the population of this study.

Selection of Cases

Patients, both male and female, in the age group of 30 to 50 years with no recent history of periodontal therapy were selected. All selected cases were free from systemic diseases, hormonal imbalances, dental caries, hypoplasia, malocclusion and other personal habits like pan chewing and smoking. They were not receiving any form of medication. The experimental site consisted of two teeth with chronic periodontitis, a pocket depth of 4 to 6 mm and a gingival index score of 2. Selected teeth were X-rayed to assess the extent of alveolar bone loss. Among those patients who satisfied the clinical criteria a sample of thirty have been selected by adopting systematic random sampling plan. Ten samples were taken in each of the three groups. The study has been designed to cover the following three groups:

- *Group I:* Irrigated with physiological saline (Control group).
- *Group II:* Irrigated with 0.2% Chlorhexidine digluconate solution.
- *Group III:* Irrigated with 0.15% Benzylamine hydrochloride solution.

Microbiological Sampling

The experimental site was isolated and the sample collected using a 1 ml, sterile insulin syringe with a disposable

23 gauge needle. The needle point was inserted to about 1 mm above the probable base of the sulcus and samples were obtained from the free unattached plaque zone. The sample was aspirated into 0.5 ml of normal saline.¹¹ A drop of the above suspension was placed on a slide and sealed with a cover slip. The suspension was then examined under a microscope fitted with a dark field condenser within 1 hour of collecting the sample. One-hundred microorganisms were counted in randomly selected fields and the percentage of Cocci, motile bacteria, spirochetes and others (which included fusiforms, filamentous forms and any organism not entering into the other three categories) were determined.¹²

Study Design

After the initial microbiological parameters were recorded in the proforma each tooth of the experimental site was irrigated with 1 ml of the following solutions.

- *Group I:* Physiological saline (Control group).
- *Group II:* 0.2 % Chlorhexidine Digluconate solution.
- *Group III:* 0.15 % Benzylamine hydrochloride solution.

The irrigation was performed with a disposable syringe and 23 gauge needle inserted to about half the depth of the pocket.¹³ Microbiological samples were collected after 24 hours, 1, 2, 3 and 4 weeks following the irrigation. Statistical evaluation of the collected data was done.

STATISTICAL ANALYSIS

Students t-test was applied to the mean value of various parameters at various time intervals with preirrigation level for the three drugs. Wherever the result is significant it is mentioned with the relevant probability level of significance. When the variation is not appreciable results are arrived by direct comparison method. Fixing the preirrigation level as 100% the various percentages are worked out and the ratio of response was arrived at.

RESULTS

The data collected were tabulated to exhibit the drug effects and time effects in two rectangular co-ordinates allocating one table each for the following parameters: (1) Spirochetes, (2) motile bacteria, (3) cocci, (4) other microorganisms.

Spirochetes

The irrigation of Saline brings down the quantum of spirochetes to 50% at the end of 1 week. The micro organisms increase after 1 week and up to the end of 4 weeks. The quantum of spirochetes present is 90% of the preirrigation level. The quantum present at the end of 1 week is statistically highly significant ($p < 0.01$) and at the end of

4 weeks is not significant. Irrigation of chlorhexidine reduces the quantum of spirochetes to 45% at the end of 2 weeks only and this reducing effect is highly statistically significant ($p < 0.01$) but the amount of spirochetes present increases upto 90% at the end of 4 weeks though not significant. Irrigation of Benzylamine brings down the amount of spirochetes present to 45% at the end of 4 weeks and this reduction is highly statistically significant ($p < 0.01$). The effect of the irrigation of Saline, Chlorhexidine and benzylamine in eliminating the presence of spirochetes upto 55% is highly statistically significant ($p < 0.01$). The results obtained at the end of 1, 2 and 4 weeks respectively indicates a ratio of response in eliminating half of the spirochetes after irrigation. This ratio can be deemed as 1:2:4 in terms of weeks. But this result is seen to be temporary because the eliminated spirochetes get revived upto 90% in the case of Saline and Chlorhexidine at the end of 4 weeks. But in the case of Benzylamine there is no question of revival of the eliminated spirochetes as the level obtained at the end of 4 weeks (45%) is highly significant. The elimination process of spirochetes set by Benzylamine is a sloping pattern and the trend slowly tapers off and complete elimination can be forecasted after 4 weeks. But this trend is not seen in the case of Chlorhexidine and Saline. In the control group and the Chlorhexidine group spirochetes decrease temporarily and then increase rapidly, the trend following a concave pattern but in the case of Benzylamine the trend is different and follows a skew type curve.

Motile Bacteria

Motile bacteria does not behave significantly due to the irrigation of Saline. Keeping the preirrigation level of saline as 100 the variation after 24 hours and the following subsequent 4 weeks is 95 to 102% and this variation is not significant. Hence motile bacteria are immune to Saline irrigation. Motile bacteria are under control by the irrigation of Chlorhexidine at the end of 24 hours and up to the end of 2 weeks. During these days, the level of motile bacteria is reduced to nearly 70% from the preirrigation level of 100%. This reduction is statistically significant ($p < 0.01$). The motile bacteria level once again goes up to 80% at the start of the third week and to 90% at the start of the fourth week. But this increase during these two periods of time is not significant. In the irrigation of Chlorhexidine the trough occurs between the end of 24 hours and the start of third week. The irrigation of Benzylamine furnishes a quite different picture in the behavior of motile bacteria. The level of motile bacteria due to the irrigation of benzylamine follows a crest and trough pattern. The level goes down to 70% at the end of 2 weeks and this level only is statistically

significant ($p < 0.05$). During the other periods of time though the variations are not significant they form a definite pattern. The pattern of variation in the levels of motile bacteria due to the irrigation of Benzylamine is similar to that of a sine curve.

Cocci

Assuming the Cocci level as 100 before the irrigation of Saline, the level attained after irrigation and at the end of 24 hours is 226 and at the end of 1 week it is 261. Thus, the Cocci level has been highly inflated and this inflation is statistically significant ($p < 0.01$) during these two periods but the Cocci level comes down during second week to 188 and the third week to 165. Cocci level during those two later periods is seen to be deflated. This deflation is statistically significant ($p < 0.05$). During the fourth week the Cocci level is 132. So the action of Saline over the Cocci level is peak during 24 hours and 1 week and tapers down till the fourth week. With the assumed preirrigation level of 100, Cocci reaches the level of 304 at the end of 24 hours, 371 at the end of 1 week, and 376 at the end of 2 weeks after irrigation with chlorhexidine. The increase is highly significant ($p < 0.01$) at all stages. The level of chlorhexidine is 223 at the end of third week and 193 at the end of fourth week. When compared with the preirrigation level, during these periods also the increase is appreciable though amount of increase is lesser than the previous part. The increase during these two periods is also highly significant ($p < 0.01$). Unlike the case of Saline the variations in the Cocci level is highly significant during the entire period of observations. It means that chlorhexidine has significant influence on the Cocci level upto two weeks only and thereafter the Cocci level slowly comes down. The level of Cocci after the irrigation of benzylamine is 273 at the end of 24 hours, 402 at the end of 1 week, 457 at the end of 2 weeks, 397 at the end of third week and 402 at the end of fourth week. Assuming the preirrigation level as base (100) the increase at all stages is highly significant ($p < 0.01$). Hence, benzylamine can be considered to have a sustained effect. On comparison the effect of Chlorhexidine is three fold and Benzylamine is four fold and thus the average ratio of response works out to be 3:4.

Other Microorganisms

Assuming the preirrigation level of saline as 100 the level of the presence of other microorganisms goes up to 150 at the end of 24 hours and then slowly comes down to the preirrigation level at the end of 4 weeks. But, all these variations are not significant. Hence, it can be reckoned that saline is neutral to other microorganisms. In the case

of chlorhexidine the presence of other microorganisms goes upto 153 at the end of 1 week and then comes down. At the end of 4 weeks, the level goes below that of the preirrigation level. Except in the case of the first week the variations are not significant. The increased level of 153 recorded during the first week is alone statistically significant ($p < 0.05$). The effect of chlorhexidine exists up to 1 week and then fades away. In the case of Benzylamine the level of other microorganisms, assuming the preirrigation level as 100, at the end of 24 hours is 229, at the end of 1 week 181, at the end of 2 weeks 233, at the end of 3 weeks 179 and at the end of 4 weeks is 168. The level goes upto and comes down and this gets repeated. The result is highly statistically significant ($p < 0.01$) up to 2 weeks and significant ($p < 0.05$) during third and fourth weeks. Even after 4 weeks, the result did not peter out to the preirrigation level. Hence Benzylamine proves to be a highly effective drug despite the sea-saw pattern it follows.

DISCUSSION

Since the primary cause of most of the periodontal diseases are bacterial in nature the rationale for treatment is based on controlling the pathogenic oral microflora among other things.⁵ Jorgen slots¹⁴ in 1979 described the subgingival microflora of healthy periodontium, gingivitis and advanced adult periodontitis. He stated that the healthy gingival sulcus harbored a scant microflora dominated by Gram-positive organisms. In 1986 FHM Mikx et al¹⁵ found that in children gingivitis is associated with increased proportions of Spirochetes. One way to prevent gingivitis and periodontitis is to control pathogenic bacterial plaque, so suggested Korman¹⁶ in 1986. The study of Lindhe, Liljenberg and Listgarten¹⁷ in 1980 found that with increasing degrees of severity of the disease the relative number of motile organisms increased while coccoid cells and straight rods decreased. In 1986 Jairam Reddy et al¹⁸ studied 52 individuals of an urban black population and found that high proportions of Spirochetes and Motile rods were present in the subgingival plaque. Cingi C et al¹⁹ in 2011 stated that Chlorhexidine gluconate and benzylamine hydrochloride mouth spray, added to standard antibiotic treatment, significantly alleviate the intensity of clinical signs in patients with streptococcal pharyngitis. The results of the study of RE Mohart et al²⁰ in 1970 suggested that bacterial metabolism may be effected by the different environmental conditions that exists in a periodontal pocket as compared to a normal gingival crevice.

The present study confirmed the results of a number of investigations according to which the periodontal pocket in periodontitis harbors a micro flora with considerable

proportions of motile bacteria and particularly Spirochetes (Listgarten et al 1978).²¹ The results of the study showed that a single subgingival irrigation with 0.2% Chlorhexidine solution or 0.15% Benzylamine solution led to a decrease in the mean percentage of spirochetes and motile bacteria with a concomitant increase in the mean percentage of Cocci and other microorganisms which is in concurrence with the study of westling et al²² 1984. It also indicated that there is a change in the subgingival flora till the end of the first week in the case of Saline, second week in the case of Chlorhexidine, before returning to near baseline levels, while Benzylamine showed a slow sustained effect till the end of 4 weeks. The results were significant. This is in accordance with the study of Lander et al¹¹ 1986 which showed a significant reduction in Motile cell numbers and Spirochetes and an increase in Cocci at 1 week after Chlorhexidine irrigation and the increase in their study was maintained up to 4 weeks. As the microbial shifts after Chlorhexidine and Benzylamine irrigations were more significant than Saline it is evident that the reduction of Spirochetes/Motile bacteria was caused by more than mere mechanical irrigation and presumably by the action of the drugs in the irrigating solution as confirmed by the study of Westling et al²² 1984.

The present study concluded that 0.15% Benzylamine hydrochloride solution was more effective than 0.2% Chlorhexidine Digluconate solution, since Benzylamine had an added advantage of being an alcoholic solution, alcohol being antimicrobial and bactericidal to all the common pathogenic bacteria. In addition studies being conducted show that an increase in environmental PH favored the drugs antiseptic action.

CONCLUSION

According to the microbiological analysis, Benzylamine can be considered as an efficient drug when compared to others on the basis of its only exceptional quality that it does not follow a concave pattern any where, i.e. it does not any where reach the preirrigation level at the end of 4 weeks.

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