Use of Membranes Containing 20% Chlorhexidine and 40% Doxycycline for Treatment of Chronic Periodontal Pockets

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Abstract

A study was carried out to compare the effects of subgingival applications of resorbable hydroxypropylmethylcellulose (HPMC) membranes containing 20% chlorhexidine (CHx) and 40% doxycycline (DOXY) placed subgingivally in periodontal pockets greater than 5 mm in depth. Each of 11 patients had at least four pockets. HPMC strips containing CHx and DOXY were inserted into each pocket twice a week for 6 w. One pocket was kept as a control and the other received subgingival scaling and root planing at 0, 1, 3 and 6 w. The microbial flora of 44 pockets from the 11 patients was examined using dark-field microscopy at the baseline and at 1, 3, 6 and 10 w. Clinical parameters including probing depth, crevicular fluid flow, bleeding on probing and gingival shrinkage were also recorded. The CHx and DOXY-administered or root-planed pockets showed marked reduction of both pocket depth and the number of motile organisms. Less bleeding was also observed in CHx and DOXY-inserted pockets.

Introduction

The importance of bacteria in the etiology of inflammatory periodontal disease has been clearly established^[1-3]. The relationship between motile organisms and the presence of disease has also been shown^[4,5]. Thus, the goal of therapy is to suppress motile organisms in particular for a clinically significant period of time. In recent years the use of sustained delivery systems for local delivery of antibacterial agents to the active site of the disease has aroused interest^[6-10]. Different materials such as monolithic fibers^[11], acrylic strips^[12] and immobilized membrane preparations have been used for controlled release of antimicrobial agents^[13,14]. Resorbable membranes can be left *in situ*, and therefore have been preferred in a recent study^[7]. Local use of antibacterial agents such as chlorhexidine, metronidazole and tetracycline has been found promising for suppressing pathogenic flora^[6,9,11,15-19]. Semisynthetic doxycycline has proved to be more active than other tetracyclines^[20,21].

The aim of this study was to compare the use of chlorhexidine(CHx) and doxycycline(DOXY) contained in resorbable membranes for the treatment of chronic periodontal pockets and to contrast their use with conventional periodontal therapy during a ten-week period.

Materials and Methods

The subjects of this study were 11 patients (8 male and 3 female; mean age 44 yr; age range 37 to 45 yr) who were diagnosed from clinical and radiographic examinations as suffering from chronic periodontitis with at least 4 periodontal pockets $\geq$ 5 mm in depth that bled after gentle probing and were associated with the mesial or distal surface of a single rooted tooth. The patients had not received dental treatment or medication within the previous 3 mon. Informed consent was obtained from all patients participating in the study. They were allocated randomly into four groups: 1) Chlorhexidine strip, 2) Doxycycline strip, 3) Root

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planing, 4) Control. After subject selection, each participant was given careful instruction in appropriate oral hygiene techniques. Supragingival calculus and overhanging margins were removed but subgingival instrumentation was not carried out. In addition, polishing of teeth was provided according to individual needs.

During the study, no further oral hygiene instruction was given to the patients, who therefore continued using their individual routine method.

At sites treated by local drug delivery, a minimum of one and a maximum of two resorbable strips were placed directly into the periodontal pockets at the level of the free gingival margin twice a week for 6 w.

The effects of different therapies were evaluated by measurement of pocket depth using a Williams periodontal probe; gingival shrinkage; sulcus bleeding index^[22] and gingival crevicular fluid^[23].

Microbiologic samples were evaluated by dark-field microscopy^[4]. A complete clinical and microbiological examination was conducted at the baseline (prior to treatment) following treatment at 1, 3, 6 and 10 w.

Preparation of Drug-containing Membranes

The HPMC (hydroxypropylmethylcellulose) powder (1.5 or 2 g) was dissolved in 38.5 ml or 38 ml of distilled water at 70°C for DOXY or CHx, respectively. The solution was then cooled to 25°C and at this temperature 0.5 g of CHx or 1 g of DOXY was added to give final drug concentrations of approximately 20 % and 40 %, respectively.

The solutions were poured into plates and kept at 37°C for 5 days in an atmosphere containing 5% CO₂ in order to obtain sheets 0.14 mm thick.

A model system was also prepared to measure them *in vitro* release of CHx and DOXY from the membranes.

In this system the concentration of drug released into the water was determined by measurement of the absorbance at 360 nm for DOXY and 253 nm for CHx in a spectrophotometer at 4, 8, 12, 14, 24, 48, 72 and 96 h.

The results of the four treatment groups were compared statistically by one-way analysis of variance (ANOVA), and serial changes within each group were assessed by paired *t* test.

Results

Cumulative percentage release of DOXY and CHx from the HPMC membranes in the *in vitro* model is shown in Fig. 1. As can be seen, the maximum release of both DOXY and CHx from the membranes took place in the first 12 h. However, the rate of release diagram (Fig. 2) showed some differences between the drugs. The release of DOXY, expressed as $\mu\text{g/h}$, in the first 8 h was higher than that of CHx. After 24 h the release of both drugs from the membranes decreased rapidly. At 24 h the rate of release was about 30 $\mu\text{g/h}$ for DOXY and 5 $\mu\text{g/h}$ for CHx. At 72 h the release of DOXY was approximately 7 $\mu\text{g/h}$, whereas that of CHx was about 2 $\mu\text{g/h}$.

DOXYCYCLINE AND CHLORHEXIDINE (HPMC)

Fig.1 Cumulative percentage release of DOXY and CHx from HPMC membranes

Fig.2 Rate of release ($\mu\text{g/h}$) of DOXY and CHx from HPMC membranes

Statistical differences between the treatments obtained by analysis of variance are summarized in Table 1. It is apparent that at the baseline there were no significant differences between the treatment groups for any of the measurement parameters. However, after treatment, significant differences were noted between the groups at various visits.

Changes in pocket depth in the different groups are given in Table 2. Intragroup analysis revealed that all the treatment groups achieved significant and gradually increasing reductions of pocket depth for up to 10 w. The control pockets showed no significant changes. Intergroup comparisons showed that all treatments reduced pocketing significantly compared with the control pockets ($p < 0.05$ - < 0.001).

A summary of statistical differences between all the groups for measurement of gingival shrinkage is given in Table 3. As can be seen, the results of intragroup analysis are similar to those for pocket depth with the exception of the first week. Intergroup analysis also revealed significant improvements compared with the control group ($p < 0.05$).

The results of intragroup analysis for measurement of the bleeding score are given in Table 4. Within the control group no significant changes in bleeding occurred. However, all the treatment groups showed significant intragroup reductions in bleeding and maintained these reductions for up to 10 w. Reductions in bleeding in the DOXY and the CHx groups were more marked than in the root planing group. At 1 w, the decreases in the bleeding scores of the treatment groups were not significant.

The results of intragroup analysis for crevicular measurements are given in Table 5. As shown in the table, crevicular fluid flow changed significantly in the treatment groups whereas no significant changes were detected in the control group with the exception of the 3rd w. Intergroup analysis, on the other hand, revealed significant differences between the groups at 6 and 10 w.

The means and standard deviations of the percentages of motiles and cocci are given in Tables 6 and 7. Intragroup analysis showed that fluctuations in the percentages of motiles and cocci were significant after only one patient visit in the control group. The differences in the other groups from the baseline up to week 10 were significant for both motiles and cocci. The decrease in the percentages of motiles and the increase in the percentages of cocci were highly significant in the treatment groups compared with the control group.

Table 1 Summary of the statistical differences between all the groups at each visit for the measurement parameters determined by analysis of variance

Week	Measurement Parameters					
	PD	GS	BL	CF	Co	Mo
0	NS	NS	NS	NS	NS	NS
1	NS	NS	NS	NS	*	**
3	NS	NS	**	NS	**	**
6	*	*	***	**	**	***
10	**	*	***	***	*	**

PD, pocket depth; GS, gingival shrinkage; BL, bleeding on probing; CF, crevicular fluid flow; Co, cocci; Mo, total motile; NS, not significant

*p<0.05, **p<0.01, ***p<0.001

Table 2 Mean and standard deviation of pocket depth in the treatment and control groups at the baseline (Visit 0) and for each visit up to 10 w (Visit 4)

Week	Patient Groups											
	Control			Root Planing			DOXY(HPMC) ^a			CHx(HPMC) ^a		
	$\bar{X}$	$\pm$	SD ^b	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD
0	6.1	$\pm$	0.7	5.9	$\pm$	1.1	6.3	$\pm$	1.5	6.5	$\pm$	1.4
1	6.0	$\pm$	0.8	5.5	$\pm$	1.3*	5.9	$\pm$	2.2*	5.8	$\pm$	1.0*
3	5.8	$\pm$	1.0	5.1	$\pm$	1.5**	5.6	$\pm$	1.8*	5.7	$\pm$	0.8**
6	5.9	$\pm$	1.1	4.6	$\pm$	1.4***	4.9	$\pm$	1.2***	5.5	$\pm$	1.2***
10	6.0	$\pm$	0.9	4.5	$\pm$	1.2***	5.0	$\pm$	1.0***	5.7	$\pm$	0.9**

a HPMC: Hydroxypropylmethylcellulose

b mean $\pm$ S.D.

Statistical analysis ; *p<0.05, **p<0.01, ***p<0.001

Table 3 Mean and standard deviation of gingival shrinkage for the treatment and control groups at the baseline (Visit 0) and at each visit up to 10 w (Visit 4)

Week	Patient Groups											
	Control			Root Planing			DOXY(HPMC) ^a			CHx(HPMC) ^a		
	$\bar{X}$	$\pm$	SD ^b	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD
0	7.7	$\pm$	2.4	7.3	$\pm$	2.6	7.3	$\pm$	2.9	7.8	$\pm$	2.2
1	7.8	$\pm$	2.6	7.5	$\pm$	2.5	7.4	$\pm$	2.3	7.9	$\pm$	2.1
3	7.9	$\pm$	2.6	7.9	$\pm$	2.5*	8.5	$\pm$	2.3**	8.8	$\pm$	1.8**
6	7.8	$\pm$	2.3	8.2	$\pm$	2.4**	8.7	$\pm$	2.6**	8.9	$\pm$	1.7**
10	7.8	$\pm$	3.1	8.5	$\pm$	2.7**	8.5	$\pm$	2.2**	8.6	$\pm$	1.7**

a HPMC: Hydroxypropylmethylcellulose

b mean $\pm$ S.D.

Statistical analysis ; *p<0.05, **p<0.01

Table 4 Mean and standard deviation of the bleeding score for the treatment and control groups at the baseline (Visit 0) and at each visit up to 10 w (Visit 4)

Week	Patient Groups											
	Control			Root Planing			DOXY(HPMC) ^a			CHx(HPMC) ^a		
	$\bar{X}$	$\pm$	SD ^b	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD
0	3.1		0.8	3.0		0.8	2.9		0.7	3.2		0.6
1	3.0		0.8	2.5		0.7	2.8		0.8	2.9		0.8
3	3.1		0.8	2.6		0.4*	2.2		0.4***	2.3		0.6***
6	2.9		0.7	1.7		0.5**	1.7		0.6***	2.2		0.5***
10	3.0		0.9	1.5		0.7**	1.6		0.7***	1.9		0.5***

a HPMC, Hydroxypropylmethylcellulose

b mean $\pm$ S.D.

Statistical analysis ; *p<0.05, **p<0.01, ***p<0.001

Table 5 Mean and standard deviation of crevicular fluid flow in the treatment and control groups at the baseline (Visit 0) and at each visit up to 10 w (Visit 4)

Week	Patient Groups											
	Control			Root Planing			DOXY(HPMC) ^a			CHx(HPMC) ^a		
	$\bar{X}$	$\pm$	SD ^b	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD
0	2.0	$\pm$	0.7	1.8	$\pm$	0.8	1.6	$\pm$	0.6	1.8	$\pm$	0.7
1	1.9	$\pm$	0.6	1.6	$\pm$	0.7	1.2	$\pm$	0.5*	1.6	$\pm$	0.8**
3	1.7	$\pm$	0.6*	1.4	$\pm$	0.7**	1.1	$\pm$	0.4**	1.6	$\pm$	0.5**
6	1.8	$\pm$	0.5	1.3	$\pm$	0.5**	1.0	$\pm$	0.4**	1.5	$\pm$	0.6**
10	2.0	$\pm$	0.6	1.1	$\pm$	0.4**	0.9	$\pm$	0.3**	1.5	$\pm$	0.7**

a HPMC: Hydroxypropylmethylcellulose

b mean $\pm$ S.D.

Statistical analysis ; *p<0.05, **p<0.01

Table 6 Mean and standard deviation of the percentage of curved and motile rods and spirochaetes in the treatment and control groups at the baseline (Visit 0) and at each visit up to 10 w (Visit 4)

Week	Patient Groups											
	Control			Root Planing			DOXY(HPMC) ^a			CHx(HPMC) ^a		
	$\bar{X}$	$\pm$	SD ^b	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD
0	29.9		10.4	28.9		12.4	28.2		12.1	30.5		10.0
1	33.5		14.7	19.0		12.6	17.1		9.3*	20.4		10.3***
3	29.6		13.9	20.0		10.1*	13.3		8.9**	17.9		8.4***
6	28.8		14.4	19.0		8.4*	11.9		10.2***	13.9		11.2***
10	31.8		10.7*	17.3		9.4**	11.0		9.6***	17.8		12.3**

a HPMC: Hydroxypropylmethylcellulose

b mean $\pm$ S.D.

Statistical analysis ; *p<0.05, **p<0.01, ***p<0.001

Table 7 Mean and standard deviation of the percentage of cocci in the treatment and control groups at the baseline (Visit 0) and at each visit up to 10 w (Visit 4)

Week	Patient Groups											
	Control			Root Planing			DOXY(HPMC) ^a			CHx(HPMC) ^a		
	$\bar{X}$	$\pm$	SD ^b	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD
0	62.1		12.5	58.9		14.8	57.3		13.9	57.4		14.4
1	58.7		12.0	68.6		13.6*	69.5		12.7*	66.0		13.2**
3	66.8		13.9	71.8		17.6*	71.9		12.1**	67.8		12.8***
6	70.7		13.1*	74.6		12.0*	73.5		11.9**	69.3		15.6**
10	62.9		10.8	76.1		10.9**	74.0		11.7**	73.5		11.2**

a HPMC: Hydroxypropylmethylcellulose

b mean $\pm$ S.D.

Statistical analysis ; *p<0.05, **p<0.01, ***p<0.001

Discussion

Methods for drug delivery to periodontal pockets have been studied by several investigators in attempts to achieve high local concentrations of antibacterial agents to aid in the suppression of periodontal pathogens^[7,14,18]. Tetracyclines have often been selected for local administration, not only because of their antimicrobial effects^[24-26] and concentration in crevicular fluid^[11], but also their fibroblast-stimulating activity^[27,28], enhancement of bone regeneration^[29,31], substantivity to mineralized tissues^[32], and anti-collagenolytic activity^[33,34].

In this study, we used hydroxypropylmethylcellulose (HPMC), a non-toxic and water-soluble polymer, as a base material. Drugs like antibiotics and antiseptics can be mixed easily with this material without affecting their original properties. HPMC containing 20% CHx and 40% DOXY was formed into solid and elastic strips. These strips were easily placed into periodontal pockets with minimal patient discomfort.

In vitro testing of CHx- or DOXY-loaded HPMC membranes indicated that maximum release of both drugs occurred within 12 h. It is desirable that the drug remain for a long period in the pocket, in order to suppress organisms that are susceptible to its effects^[6,8].

In our *in vitro* model, the rate of release at 24 h was around 30 μ g/h for DOXY and 5 μ g/h for CHx. In an *in vitro* study with HPMC strips loaded with 1% tetracycline or 5% CHx, Noguchi et al.^[7] showed that almost 100% of the impregnated drug was released within 24 h. Goodson et al.^[11] described the preparation of a biodegradable polycaprolactone containing tetracycline fiber, which released nearly all its drug content in 1 day *in vitro*. The differences between the results may be due to the type, properties and thickness of the polymer.

Changes in clinical parameters such as reduction of pocket depth, bleeding on probing and gingival shrinkage were observed in the DOXY, CHx and root planing groups. After 10 w, reduction of bleeding on probing was significantly greater in the pockets of the DOXY and chlorhexidine groups compared to the root planing group. Such effects were also noted in the study of Addy et al.^[12], who compared acrylic strips containing metronidazole, chlorhexidine and tetracycline with root planing. Metronidazole and root planing produced immediate effects on microbiological and clinical parameters, most notably a reduction in pocket depth, which in many cases persisted for 14 w after treatment.

However, in our study, the prevalence of spirochetes and motile rods was markedly reduced in both DOXY- and CHx- administered pockets for 3 to 10 w.

The reason for the clinical differences between DOXY and CHx is not clear, but may be due to the specific biological properties of DOXY such as its concentration in the gingival crevicular fluid. Our findings

have also been supported by the results of another clinical *in vivo* study, by Heijl et al.^[35], who compared the effectiveness of tetracycline-containing drug delivery fibers and scaling after a period of 62 d. Fiber therapy with or without scaling reduced bacterial counts by approximately two orders of magnitude. According to these results, the combination of fiber therapy with scaling eliminated bleeding on probing and produced the greatest mean reduction in pocket depth.

In conclusion, the results of our study indicate that HPMC strips containing 40% DOXY and 20% chlorhexiaire strips have significant effects and benefits on clinical and microbiological parameters in gingival pockets more than 5 mm deep in a 10-week period.

Further investigations are needed to improve controlled drug delivery into periodontal pockets for prolonged periods using suitable base materials and minimal dosage, but giving maximum concentrations.

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