DRUG DEVELOPMENT RESEARCH 70 : 395–401 (2009)

DDR

Research Article

The Evaluation of a Biodegradable Dental Chip Containing Chlorhexidine in Chitosan Base as a Targeted Drug Delivery in the Management of Chronic Periodontitis in Patients

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Chlorhexidine is one of the commonly used agents in the treatment of periodontitis. In the present study, a biodegradable dental chip of chitosan containing chlorhexidine was evaluated both in vitro and in vivo, as a targeted drug delivery system in patients of chronic periodontitis. Thirty patients having localized periodontal pockets ≥ 5 mm were selected. At baseline, the experimental patients received full mouth scaling and root planing followed by placement of a chlorhexidine chip. The placebo group received plain chitosan chips, and conventional scaling and root planing were performed for the control group. Measurements of plaque, gingival index were recorded at 1, 2, 3, and 4 months. Probing depth and clinical attachment levels were recorded at the 3rd and 4th month. GCF and saliva samples were procured from the subjects to analyze the drug release at 0, 2, 4, 6, and 24 h and on days 2, 3, 5, 7, 9, 11, 13, 15, 30, 60, and 90. The study showed improvement in plaque index, gingival index, probing depth, and clinical attachment levels, but the chlorhexidine chip–treated group showed a significantly better improvement than placebo and control groups at the end of 120 days. Based on the results of the present in vivo study, we can conclude that the chlorhexidine-containing chitosan drug delivery system may be an adjunct in treating patients with chronic periodontitis. Drug Dev Res 70 : 395–401, 2009.

Key words: chlorhexidine; chitosan; chronic peridontitis

INTRODUCTION

Conventional periodontal therapy has been targeted to altering the periodontal environment to one that is less conducive to retention of bacterial plaque [Soskolne et al., 1998]. With increased acceptance of the role of specific periodonto pathogens in the etiology of periodontal disease, antimicrobial therapy has established itself as an adjunct to the traditional mechanical form of therapy. Treatment modalities have evolved directed towards specific sites thereby changing from a systemic therapeutic trend to a more localized form of treatment. Local drug delivery systems have been used in various controlled release

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Received 25 December 2008; Accepted 4 February 2009

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ddr.20316

forms from non-degradable devices to presently available bio-degradable devices [Goodson et al., 1980]. Though these agents showed clinically beneficial effects, the potential for development of bacterial resistance led to a renewed interest in the commonly used topical antibacterial, chlorhexidine Chlorhexidine, a bisbiguanide used as an antiplaque agent that has been recently evaluated in local delivery devices for the treatment of periodontal pockets [Quirynen et al., 2001]. Periochip™ a commercially available biodegradable chlorhexidine formulation with a gelatin base, has shown improvement in clinical parameters [Killoy, 1998]. Additionally, polymers like ethyl cellulose, polylactic glycolic acid, and gelatin are being used as a base in pharmaceutical preparations [Singla and Chawla, 2001].

Chitosan a natural polysaccharide processed from chitin, a derivative of the exoskeleton of arthropods, has antimicrobial, anti-inflammatory, wound healing, and mucoadhesive properties suggestive of potential in local drug delivery systems. In the present study, the sustained release of chlorhexidine from a chitosan-based chip was assessed both in vitro and in vivo.

MATERIALS AND METHODS

Preparation of the Biodegradable Chitosan Chip

Chitosan was soaked in 1% acetic acid and kept overnight. It was then dissolved in water, sonicated to provide a homogenous mixture, and poured into specially designed rectangular glass molds lined with aluminum foil. After drying overnight at room temperature, the resultant film was cut into small rectangular chips of size 0.5×0.5 sq cm. These were wrapped in aluminum foil and stored in sterile vials at room temperature. The thickness of the biodegradable chip was standardized to be 0.52 ± 0.33 mm.

Preparation of Chlorhexidine Incorporated Biodegradable Chitosan Chip

Chlorhexidine (2.5 mg; 15% w/w) was dissolved in solvent and added to the chitosan that was soaked in 1% acetic acid overnight. Both ingredients were sonicated to provide a homogenous mixture and poured into specially designed rectangular glass molds lined with aluminum foil. After drying overnight at room temperature, the resultant film was cut into small rectangular chips of size 0.5×0.5 sq cm. A content uniformity test was done on a few chips randomly to confirm the exact amount of drug dispensed in each chip. The chips were then placed in sterile vials and stored at room temperature.

In Vitro Release Study

A "vial" method was employed for the in vitro release study. Chips of 0.5×0.5 sq cm were placed in glass vials containing 5 ml of phosphate buffer saline. Samples (1.0 ml) were withdrawn periodically at an interval of 2 up to 6 h and at 1, 2, 3, 5, 7, 9, 11, 15, and 30 days, each time replacing the sample with the equivalent of fresh phosphate buffer saline to maintain sink conditions. The samples were analyzed spectrophotometrically at 254 nm for the drug. The concentration of chlorhexidine was calculated from the calibration curve prepared in phosphate buffer saline. An in vitro drug release plot of time versus cumulative percent drug release was constructed from the data obtained.

In Vivo Release Study

Subjects

Thirty patients with chronic periodontitis (17 females and 13 males) ≥ 30 years of age with a minimum of 20 natural teeth who had localized periodontal pockets of ≥ 5 mm either in the maxillary or mandibular arches were selected. Patients were excluded if they were pregnant, had systemic disease, a history of allergy to chlorhexidine, or a history of antibiotic treatment within the past 6 months. After screening examination, qualifying patients received consent forms. Approval for the study was granted by the Institutional Board of Manipal University.

Clinical Trial Design

At the initial visit, scaling and root planing were done with emphasis on brushing using a Modified Bass technique [Poyato-Ferrera et al., 2003] and oral hygiene maintenance. Patients were then recalled after 2 weeks for review. Only those patients with residual pocket probing depth ≥ 5 mm were included in this study based on the specific inclusion and exclusion criteria. The details of the selected patients were recorded in a standard proforma.

Procedure

Patients were divided into three groups of 10 each. The first group was administered a chlorhexidine chip (drug group), the second group was given a plain chitosan chip (placebo group), and the third group served as the control group in which only scaling and root planing were done. Patients were randomly allocated into these 3 groups. Baseline measurements included plaque index [Silness and Loe, 1964], gingival index [Loe and Silness, 1963], pocket probing depth, and clinical attachment level. Occlusal stents were made to standardize the probe placement into the pockets and to ensure reproducibility at every visit. For the drug and placebo group, a rectangular chip $0.5 \times 0.5\,cm^2$ was carefully inserted into the pocket with a probing depth ≥ 5 mm using a cord-packing instrument. Once the chip

came into contact with the gingival crevicular fluid (GCF), it swelled up and adhered to the lateral wall of the pocket due to mucoadhesive properties of the chitosan. To study the in vivo drug release profile, GCF and saliva samples were collected at 0, 2, 4, and 6 h. A Whatman no. 1 filter paper disc ($0.5 \times 0.5\,cm^2$) was then carefully introduced into the sulcus of the experimental tooth with a tweezer and left in position for 3 min, at the end of which the filter paper disc was transferred into vials containing 5 ml phosphate buffer solution. Similarly, for collection of saliva samples, two filter paper discs were placed on the floor of the mouth for 3 min, after which they were transferred into separate vials containing 5 ml phosphate buffer solution. The vials containing the samples were closed, vigorously shaken for 2 min, and then left undisturbed for 10 min to ensure that the entire drug quantity absorbed was extracted from the filter paper. The resultant solution was filtered to remove impurities like blood or plaque and analyzed using the Hewlett Packard UV spectrophotometer to obtain absorbance of the drug. The mean concentration values of drug absorbed from GCF and saliva were calculated.

The patients returned the next day and consequently on days 2, 3, 5, 7, 9, 11, 13, and 15 for the collection of GCF and saliva samples. They were asked to refrain from the use of mouthwashes, dental floss, and interdental cleansing aids for a period of one month to avoid dislodgement of the chip. Each patient was advised to report to the clinic in case of any adverse effects to the material used. The 4-month study trial involved monthly visits in which the first and second month involved only recording of plaque and gingival index. No attempts were made to record pocket probing depth or attachment loss to avoid disturbing pocket healing. GCF and saliva samples were again collected. In the third and fourth month, all clinical parameters were measured including pocket-probing depth and clinical attachment level.

Statistical Analysis

Changes in plaque index, gingival index, pocket probing depth, and clinical attachment levels between baseline and 4 months within the treatment groups were analyzed using the Wilcoxon's signed rank test (an alternative to paired t-test).The Mann-Whitney Test (an alternative to an unpaired t-test) was used to determine significant differences between the drug, placebo, and control groups.

RESULTS

In Vitro Drug Release Profile From the Chip

In vitro release studies of drug delivery systems are important property for the characterization of the system. With the help of in vitro drug release studies in simulated in vivo conditions, the performance of the system in in vivo conditions can be predicted. The release pattern was biphasic characterized by an initial burst release of around 20% for 24 h followed by a slow release for a period of 30 days (Fig. 1). The mean concentration of chlorhexidine gluconate in 5 ml phosphate buffer saline (pH 7.4) was found to be 0 μg/ml at commencement of the trial and increased to a mean concentration of 159.1 ± 1.01 μg/ml by the second hour. At the end of 24 h, the mean drug concentration was 94.25 ± 0.08 μg/ml, which then decreased to 30.16 ± 0.20 μg/ml over a period of 30 days. At the end of this period, a cumulative drug release of 94.45% was observed (Fig. 1).

In Vivo Drug Release Profile in GCF and Saliva

No adverse clinical effects occurred in any of the three study groups. Analysis of GCF and saliva showed a biphasic drug release pattern with an initial burst of around 17% for the first 24 h followed by slow release of the drug up to 30 days (Fig. 2). The mean concentration of chlorhexidine in GCF was

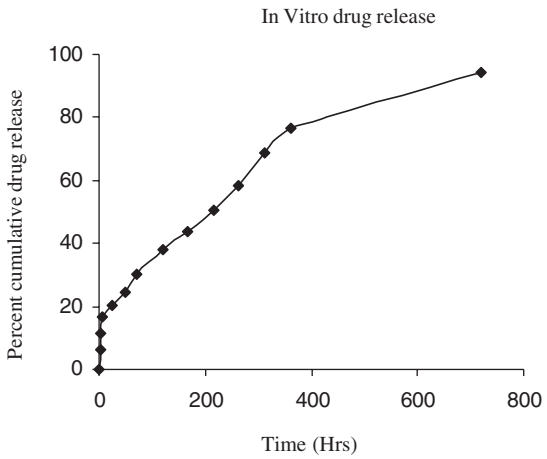

Fig. 1. In vitro drug release.

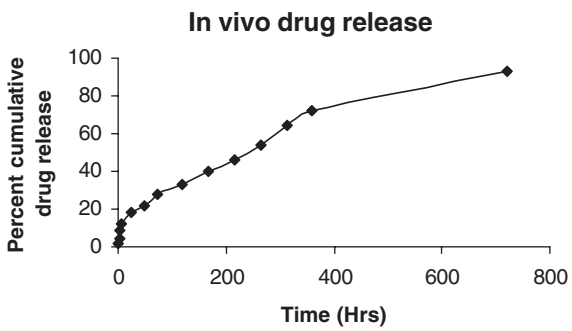

Fig. 2. In vivo drug release.

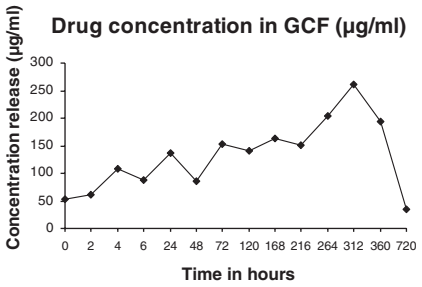

Fig. 3. Drug concentration in GCF (µg/ml).

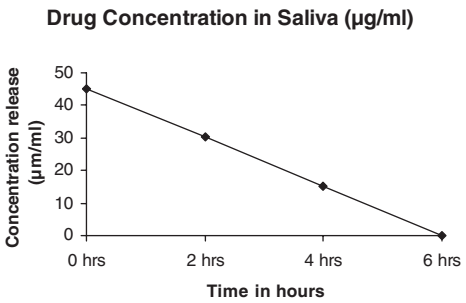

Fig. 4. Drug concentration in saliva (µg/ml).

52.5 ± 1.05 µg/ml at the time of chip insertion, reaching a minimum of 34.5 ± 1.50 µg/ml at the end of 30 days (Fig. 3). A cumulative drug release of 92.86% was evident at the end of the month (Fig. 2).

The mean concentration of chlorhexidine in saliva was 45.1 ± 0.5 µg/ml soon after chip insertion, dropping to 30.2 ± 0.3 µg/ml at hour 2. At the end of hour 4, only 15.2 ± 0.04 µg/ml was observed. At the end of 24 h, drug levels could not be detected by UV spectrophotometry (Fig. 4). A more sensitive High Performance Liquid Chromatography assay may be required to detect compound at these levels.

Oral hygiene status was evaluated by measuring plaque and gingival indices during the 4 monthly visits. All 3 groups showed good oral hygiene with a significant reduction from baseline to the 4th month (Table 1). There was no reduction in gingival inflammation between placebo and drug groups ($P = 0.189$) (Table 2) while all groups showed a reduction in probing depth from baseline to the 4th month (Table 1). The reduction in probing depth was significantly different between the drug group and the placebo and control group but there was no difference between the placebo and control groups (Table 2). Similarly, an improvement in clinical attachment level was observed in all 3 groups from baseline to the 4th month (Table 1) with the drug group showing a statistically significant gain in attachment versus both the placebo and control groups. The gain in attachment was not different between the placebo and control groups (Table 2).

DISCUSSION

The use of local drug delivery systems is gaining increased acceptance as a treatment modality in the management of chronic periodontitis. Several agents have been evaluated for this purpose with varying results. The present randomized, parallel, double-blind, 120-day trial was designed to evaluate the benefits of use of chlorhexidine in a chitosan base controlled-release chip as an adjunct to scaling and root planing and also to evaluate the release pattern of chlorhexidine in vitro and in vivo.

The in vitro release study was conducted prior to the commencement of the clinical trial in an environment comparable to in vivo conditions, so that the execution of the biodegradable system in the pocket environment could be predicted. This would also aid to optimize the drug to obtain the desired release characteristics. GCF and saliva samples were collected at various time intervals and were analyzed for drug content using a standard plot of chlorhexidine gluconate that was constructed from the data obtained by in vitro studies.

The results showed a "biphasic controlled release" with an initial burst of 17% in the first 24 h followed by a release until the end of 30 days (Fig. 2). The initial burst was attributed to the unbound chlorhexidine present at the surface of the device and the slow release of drug occurring by diffusion via an erosion induced pore enlargement. The morphological changes of the polymer and a reduction in the concentration gradient could also have contributed to the decreased rate of drug dissolution in the polymer. The mean concentration of chlorhexidine in GCF was in the range of 61 ± 1.50–194 ± 1.50 µg/ml up until the 15th day. This was well above the minimum inhibitory concentration of 125 µg/ml required to inhibit 99% of bacterial flora isolated from periodontal pockets [Stanley et al., 1989]. The secondary slow release as seen in vitro may be responsible for the decrease of the drug concentration in GCF after the 15th day. A minimal drug concentration of 34.5 ± 1.50 µg/ml (Fig. 3) was evident even by the 30th day. The in vivo release profile of chlorhexidine from Periochip™ showed a mean concentration of drug release that peaked to 2,007 µg/ml at 2 h post-chip-insertion with a slightly lesser concentration of 1,300–1,900 µg/ml being maintained over 96 h. The concentration then progressively decreased to 57 µg/ml at 9 days after chip insertion [Soskolne et al., 1998]. The results obtained in the present study were different as there was a gradual and uniform release of the drug into the GCF,

TABLE 1. Comparison of Clinical Parameters Between Baseline Values and 4th Month

Group	Clinical parameters	Mean	SD	z value[a]	*P* level	Significance
Placebo	*Plaque index*					
	Baseline	0.75	0.26			
	4 M	0.53	0.25	2.17	0.030	Significant
	Gingival index					
	Baseline	1.25	0.44			
	4 M	0.63	0.27	2.72	0.007	Significant
	Probing depth reduction					
	Baseline	6.70	1.30			
	4 M	5.60	1.00	2.6	0.009	Significant
	Probing attachment gain					
	Baseline	6.40	1.10			
	4 M	5.50	1.00	2.46	0.014	Significant
Drug	*Plaque index*					
	Baseline	0.78	0.22			
	4 M	0.33	0.24	2.72	0.007	Significant
	Gingival index					
	Baseline	1.28	0.36			
	4 M	0.47	0.35	2.82	0.005	Significant
	Probing depth reduction					
	Baseline	6.90	0.70			
	4 M	3.80	0.90	2.84	0.005	Significant
	Probing attachment gain					
	Baseline	6.60	1.00			
	4 M	3.60	0.70	2.84	0.005	Significant
Control	*Plaque index*					
	Baseline	0.83	0.21			
	4 M	0.72	0.29	2.11	0.035	Significant
	Gingival index					
	Baseline	1.40	0.38			
	4 M	0.87	0.35	2.87	0.004	Significant
	Probing depth reduction					
	Baseline	6.70	1.20			
	4 M	5.70	1.20	2.43	0.015	Significant
	Probing attachment gain					
	Baseline	6.10	1.20			
	4 M	5.60	1.30	2.24	0.025	Significant

[a]Wilcoxon's Signed Rank Test.

which was maintained over a period of 15 days with concentrations well above the minimum inhibitory level (125 μg/ml). Further, there was a gradual decline in the concentration that tapered off only by the 30th day. The mean concentration of the drug in saliva in the present study was 45.1 ± 0.5 μg/ml at the time of chip insertion, which decreased to 15.2 ± 0.04 μg/ml by the 4th hour.

The clinical trial design involved assessment of parameters of plaque index, gingival index, probing depth, and clinical attachment levels. Plaque index was taken to monitor the patient's oral hygiene status throughout the trial period. A significant reduction in plaque scores was seen in all 3 groups (drug, placebo, and control) from baseline to the 4th month (Table 1). Of the 3 groups, only the drug group showed a significant reduction in plaque scores compared to the control ($P = 0.003$) and placebo groups ($P = 0.056$) (Table 2). This could be as a result of the effect of chlorhexidine in addition to the regular maintenance by the patients. These findings are in agreement with those of Heasman et al. [2001] where patients receiving a chlorhexidine chip as an adjunct to scaling and root planing showed a reduction in plaque scores. All subjects participating in the present study showed improvement in their gingival status from baseline to the 4th month (Table 1). Between the 3 groups, a statistically significant reduction in gingival inflammation was observed only between the drug and control group ($P = 0.037$) (Table 2). These results are comparable to the studies of Jeffcoat et al. [1998] and Heasman et al. [2001].

The data from the present study also showed a significant reduction in the probing depths in all 3

TABLE 2. Comparison of Changes in Clinical Parameters Between Baseline Values and the 4th Month

Group	Clinical parameters	Mean	SD	*P**	Significance
Placebo vs. drug	*Reduction in plaque scores*				
	Placebo	0.22	0.25		
	Drug	0.45	0.23	0.056	Significant
	Reduction in gingival inflammation				
	Placebo	0.62	0.34		
	Drug	0.81	0.39	0.189	Not significant
	Reduction in PD				
	Placebo	1.1	0.7		
	Drug	3.1	1.0	0.001	Significant
	Probing attachment gain				
	Placebo	0.9	0.7		
	Drug	3.0	0.8	0.0003	Highly significant
Placebo vs. control	*Reduction in plaque scores*				
	Placebo	0.22	0.25		
	Control	0.11	0.16	0.211	Not significant
	Reduction in gingival inflammation				
	Placebo	0.62	0.34		
	Control	0.53	0.14	0.533	Not significant
	Reduction in PD				
	Placebo	1.1	0.7		
	Control	1.0	0.8	0.807	Not significant
	Probing attachment				
	Placebo	0.9	0.7		
	Control	0.5	0.5	0.224	Not significant
Drug vs. control	*Reduction in plaque scores*				
	Drug	0.45	0.23		
	Control	0.11	0.16	0.003	Significant
	Reduction in gingival inflammation				
	Drug	0.81	0.39		
	Control	0.53	0.14	0.037	Significant
	Reduction in PD				
	Drug	3.1	1.0		
	Control	1.0	0.8	0.001	Significant
	Probing attachment gain				
	Drug	3.0	0.8		
	Control	0.5	0.5	0.0001	Highly significant

*Mann-Whitney Test.

groups from baseline to the 4th month (Table 1) with the drug group showing a significant reduction in probing depth ($\geq$2 mm) compared to the placebo and control ($P = 0.001$) (Table 2). The combined effect of controlled release chlorhexidine following scaling and root planing and the antimicrobial properties of chitosan may be the reason for greater reduction in probing depth in the drug group. Soskolne et al. [1997] observed a reduction in probing depth in pockets $\geq$7 mm, for their chlorhexidine chip group at the 3rd month, followed by further improvement in probing depth at the 6th month. Jeffcoat et al. [1998] and Heasman et al. [2001] in two separate studies have shown greater reduction in probing depth with scaling root planing and chlorhexidine chip when compared to scaling and root planing alone.

In the present study, all 3 groups showed significant gain in attachment from baseline to the 4th month of observation (Table 1) with the drug group showing a significant gain in attachment ($\geq$2 mm) compared to the placebo and control groups (Table 2). Similar findings were reported by Soskolne et al. [1997] who observed an improvement in clinical attachment levels in the test group compared to the group receiving only scaling and root planing. Similarly, Stabholz et al. [1991] achieved an attachment gain of $\geq$3 mm in a 2-year clinical trial using a chlorhexidine chip.

During the 4-month trial, parameters of plaque and gingival indices were recorded at monthly intervals with probing depth and clinical attachment levels being recorded from the 3rd month onwards to avoid disturbing the pocket healing [Greenstein, 1992; Staffileno et al., 1968]. The clinical probing depth did not differ between the 3rd and 4th months. Incidentally, Stabholz et al. [1986] reported that the

antimicrobial effect of locally delivered controlled release chlorhexidine chip is sustained for up to 11 weeks after administration. Similarly, Jeffcoat et al. [1998] also advocated the 3-month interval for re-administration
of chlorhexidine chip in case of residual pockets following treatment as not much improvement was observed beyond this time period. This would probably be clinically beneficial as an alternative treatment for residual pockets seen during a maintenance phase.

This present clinical trial was conducted to evaluate the adjunctive use of an indigenously prepared biodegradable chitosan polymeric matrix incorporated with chlorhexidine (2.5 mg), used as a local drug delivery system in the management of chronic periodontitis. The following observations were made:

- In vivo drug release profile was comparable to the in vitro study.
- Chlorhexidine incorporated in chitosan base used as a targeted drug delivery enhances the benefits of scaling and root planing.
- Chitosan polymer, the vehicle for drug delivery, was effective for controlled drug release.

Based on these observations, we can conclude that the targeted drug delivery system using chlorhexidine with chitosan base polymer has been observed to be a clinically safe and effective treatment option in achieving reduction in probing depths and gain in clinical attachment levels. Therefore, it could be considered as an alternative modality for the management of patients with chronic periodontitis especially during the maintenance recall intervals. The decision to use local drug delivery during the active treatment or maintenance should be based upon clinical findings, responses to therapy, and desired clinical outcomes [Greenstein, 2006].

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