Bioerodible Injectable Poly(ortho ester) for Tetracycline Controlled Delivery to Periodontal Pockets: Preliminary Trial in Humans

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ABSTRACT The semisolid consistency of poly(ortho esters) (POEs) containing tetracycline free base allows direct injection in the periodontal pocket and shows sustained and almost constant in vitro release in phosphate buffer, pH 7.4 at 37°C, for up to 14 days. Total polymer degradation concomitant with drug release was obtained. Formulations containing 10% or 20% (wt/wt) tetracycline were evaluated in a panel of 12 patients suffering from severe and recurrent periodontitis. In the first trial including 6 patients, single-rooted teeth and molar teeth with furcations were treated immediately after scaling and root planing. Patients tolerated both formulations well, experienced no pain during application, and showed no signs of irritation or discomfort during the observation period. However, retention of the formulation was minimal in this first study. An improved clinical protocol followed in the second study (stopping bleeding after scaling and root planing) prolonged the retention of the formulations in the inflamed periodontal pockets. For up to 11 days, tetracycline concentrations in the gingival crevicular fluid were higher than the minimum inhibitory concentration of tetracycline against most periodontal pathogens.

KEYWORDS: Periodontitis, poly(ortho esters), bioerodible polymers, tetracycline, controlled release, sustained delivery system.

INTRODUCTION Periodontal diseases are a group of inflammatory and localized microbial-induced infections involving the supporting tissues of the teeth, the gingiva, periodontal ligament, and alveolar bone. Inflammation of the gingiva is referred to as gingivitis, whereas extension of inflammation into deeper tissues accompanied with bone loss is termed periodontitis. In patients with periodontitis, conventional mechanical periodontal therapy can improve the overall gingival health and,

generally, halts the progression of disease.¹ In some periodontal sites, however, gingival inflammation persists or recurs and periodontal attachment loss progresses despite gingival scaling and root planning (SRP).^{2,3} If no additional treatment is given as an adjunct to SRP in recurrent sites, periodontal attachment loss may further advance and, ultimately, result in premature tooth loss. Failure of conventional periodontal therapy may be related to an incomplete elimination of periodontopathic bacteria.^{4,5} Due to the bacterial etiology of recurrent, or refractory periodontitis, antimicrobial agents are currently regarded as the first choice for adjunctive therapy of these diseases.⁶ Systemically applied antimicrobials have been advocated for the treatment of periodontitis.⁷⁻⁹ However, drawbacks such as severe side effects, bacterial resistance, and poor local concentrations of antimicrobials have limited this route of administration.^{10,11} Local therapy provides much higher drug concentrations at the diseased site with lower total doses than systemic treatment does. This minimizes the occurrence of systemic side effects and bacterial resistance.

While the removal of all the bacterial deposits in pockets deeper than 4 mm is a difficult if not an impossible task, studies have shown that it is possible to obtain a measurable clinical effect by maintaining antibacterial activity in the periodontal pocket for approximately 1 week.^{6,12} Several polymeric systems for antibiotic delivery have been studied and evaluated for the treatment of periodontal diseases. A general review summarizing various local adjunctive treatments for periodontitis based on biodegradable or nonbiodegradable polymers has recently been published.¹³ Three particular problems common to many drug delivery systems designed for periodontal application are the nondegradability requiring a second visit to the dentist to remove the device,^{14,15} the poor retention in the periodontal pocket,^{16,17} and the difficult as well as time-consuming application of the delivery system.¹⁸ These problems may be resolved by using bioerodible, injectable, and adhesive polymers such as semisolid poly(ortho esters) (POEs) as carriers for the antimicrobial agent.

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Auto-catalyzed POEs (POE_xLA_y) are a member of a new family of POE able to degrade predominantly by surface erosion and then to sustain drug release for days to weeks, depending on their physicochemical properties related to the percentage of lactic acid in the polymeric backbone.¹⁹⁻²³ Alternatively, low molecular weight POE₇₀LA₃₀ with low viscosity and low molecular weight dispersion were synthesized by using decanol as a chain stopper and were well characterized.²⁴ The polymers reproducibly obtained are easily injectable and are therefore suitable for the preparation of the antimicrobial delivery system for periodontal application.

Formulations based on semisolid auto-catalyzed POE₇₀LA₃₀, containing tetracycline free base (TB), were evaluated in vitro in a previous study.²⁵ TB incorporated into these materials was released within 10 to 14 days depending on polymer structure. Increase in lactic acid content in the polymer tended to increase the drug release rate and to reduce the initial lag time. Tetracycline release from such a bioerodible delivery system occurs predominantly by surface erosion of the polymeric matrix, leading to kinetics, which can be zero order. These formulations loaded with TB 10% or 20% showed complete in vitro degradation concomitant with drug release.

The objective of the present study is to evaluate the injectability and the retention of the POE/TB formulations in the periodontal pockets by measuring tetracycline concentrations in gingival crevicular fluid (GCF). The in vitro tetracycline release profile was evaluated beforehand. The periodontal effect and the tolerance of these bioerodible injectable formulations after a single application were also evaluated. In addition, the role of the clinical protocol in the success of the periodontal therapy is also underlined in the current study.

MATERIALS AND METHODS

Materials

Low molecular weight auto-catalyzed POExLA_y, in which LA stands for lactic acid and y for the molar ratio of lactic acid units, were synthesized by the acid-catalyzed condensation of 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5,5]undecane with 1,10-decanediol, 1,10-decanediol-dilactate and n-decanol. Low molecular weight oligomers, unreacted monomers, and the catalyst were removed by dissolution-precipitation using THF and methanol as solvent and nonsolvent. The precipitate was dried under vacuum at 40°C for 48 hours. A self-catalyzed POE containing 30 mol% of lactic acid units (POE₇₀LA₃₀, Mw=5200, Mn=3400, I=1.53) was selected for this study because of its viscous state and degradation period. Synthesis and degradation studies of this polymer were described earlier.^{19,21,24}

TB was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The controlled release systems were prepared by simple mixing of the semisolid POE₇₀LA₃₀ and the drug without using organic solvent as described earlier.²⁵ Gamma irradiation was performed using an 18 000 Ci activity ⁶⁰Co source (Federal Research Institute, Wädenswil, Switzerland). The dose rate and total radiation dose were set at 0.8 kGy/h and 20 kGy, respectively. The samples were irradiated under argon at -70°C. Two tetracycline loading 10% and 20% (wt/wt) were evaluated.

In vitro drug release was conducted in specially thermostated cells at 37°C circulated with phosphate buffer.²⁶ The amount of drug released was measured by high-performance liquid chromatography (HPLC) using the method described previously.²⁵

Patients

Twelve healthy adult patients participated in the study. The baseline admission criteria were the following: no history of diabetes, rheumatic fever, blood dyscrasia, or immunology anomalies; no exposure to antibiotics within the previous 6 months; no long-term exposure to anti-inflammatory medications; no hypersensitivity or allergy to tetracycline or POE; no periodontal treatment within 1 year; and presence of 4 or more residual pockets > 5 mm. Subjects were informed about the aim of the study and possible side effects. The study was approved by the ethical committee of the School of Dental Medicine (Geneva, Switzerland) and a consent form was obtained from each participant.

Clinical protocols

Before the beginning of the study, all patients received hygiene instructions and were able to maintain a good level of hygiene. Four sites were selected on the basis of clinical criteria (accessibility, probing depth > 5 mm). Selected sites were scaled subgingivally using ultrasonic scalers and hand-curettes, were isolated from saliva contamination with cotton rolls, and were air dried. The patients were consecutively assigned to 2 different clinical protocols.

Clinical protocol A: Six patients were included. Four single-rooted teeth were treated in each of the first 3 subjects, and at least 2 molar teeth with furcation involvement were treated in the other 3 patients. The study sites were scaled and root placed before the application of the formulation containing TB 10% or 20% (wt/wt). The 2 formulations were tested simultaneously in the same patient.

Clinical protocol B: Six patients were included. Prior to the placement of the formulation into the pocket, a retraction cord (Gingibraid[®] Van R, Kaladent, Geneva, Switzerland) was inserted into the pocket by using a spatula (**Figure 1**). The retraction cord was left in the pocket for 15 minutes in order to achieve hemostasis. Immediately after the removal of the retraction cord, the formulation POE/TB 20% (wt/wt) was placed directly into the periodontal pocket.

Figure 1. The retraction cord Gingibraid® is inserted into the pocket by using a spatula.

The viscous formulation was injected using a syringe designed for intraligamentar anesthesia (Ligmaject®, Henke-Sass, Wolf GmbH, Tuttlingen, Germany) (**Figure 2**). The pocket was filled until the formulation could be seen at the gingival margin, and the excess was eliminated (**Figure 3**). Approximately 20 mg to 30 mg of the formulation were injected depending on the depth of the pocket.

Figure 3. Typical appearance of a periodontal site after the administration of the delivery system POE/TB.

Figure 2. Ligmaject® syringe used for the injection of the delivery system.

In order to assess the tolerance and the retention of the formulations, the patients were examined at baseline (day 0), just before the scaling and the application of the product, and then at 3, 5, 7, and 10 days (group A) or 4, 7, and 11 days (group B) after the placement of the antibiotic product. The tolerance of the formulations was evaluated by visual inspection of the gingiva (color and form) and by the presence of signs such as pus or pain. Observations were recorded for each site.

The retention time of the formulation was determined indirectly by measuring the concentration of tetracycline in GCF using a bioassay. The sites were isolated and dried by a gentle stream of compressed air. After 3 minutes, GCF was sampled using paper strips (Periopaper®, Oraflow Inc., New York, USA) placed at the gingival margin of the test sites for 30 seconds (**Figure 4**), and the volume of GCF was determined by a fluid analyzer (Periotron® 8000, Oraflow Inc., New York, USA). The strips were then placed in a petri dish onto a surface of agar (Muller-Hinton 2, Oxoid GmbH, Wesel, Germany) that had been previously inoculated with a strain of *Bacillus cereus* (ATCC 11778). After 24 hours of incubation at 37°C, the diameter of inhibition around the strip was measured (**Figure 5**). The quantity of tetracycline on each strip was determined according to a standard curve. The concentrations were then obtained by dividing the tetracycline quantity by the volume of GCF collected on that sample strip.

Figure 4. Sampling of GCF in a periodontal pocket using a paper strip.

Figure 5. Determination of tetracycline in GCF by growth inhibition bioassay.

Probing pocket depth (PPD), bleeding on probing (BOP), recession (R), and presence or absence of pus were recorded before the scaling at day 0 and at day 10 (group A) or day 11 (group B) at the completion of the observation period. The patients were thereafter treated according to their needs.

RESULTS AND DISCUSSION Figure 6 shows the data of the in vitro release of tetracycline from POE₇₀LA₃₀. For both drug loading, tetracycline was continuously released for up to 2 weeks without burst effect. In a first phase, a daily 10% of total TB was released during the first 5 days. In a second phase, daily TB released was approximately 5%. It has been reported that about 0.48 mL of GCF is produced per day in a 5-mm periodontal pocket.¹² From these in vitro results, it is expected that local concentration of TB would be higher than 1-2 µg/mL which is the minimum inhibitory concentration (MIC) of tetracycline against the majority of periodontal pathogens.

Figure 6. Cumulative in vitro release of tetracycline from POE₇₀LA₃₀ (○) 10% and (●) 20% (wt/wt); PBS pH 7.4; 37°C; n = 3, mean ± SD.

Study A:

Both formulations were well tolerated with no pain during application, and no signs of irritation, discomfort, or suppuration after treatment.

Figure 7 illustrates the TB concentrations in the GCF at days 3, 5, and 7 following the injection of the formulations. Concentrations well above the MIC of most pathogens were seen for at least 7 days in some sites confirming that the formulations are retained in the periodontal pocket for a prolonged period, releasing a sufficient amount of tetracycline to eliminate pathogenic bacteria. However, only 15% of treated sites showed prolonged retention time, and no difference between both drug loading was noticed.

Figure 7. Study A. Tetracycline concentrations (mean ± SD) in positive sites after the placement of the delivery system.

The early clinical response to the treatment with the POE₇₀LA₃₀/TB systems was evaluated at day 10. Changes in clinical parameters are summarized in **Table 1**.

Table 1. The Influence of the Clinical Experimental Design on the Change in Clinical Parameters for Sites Treated with POE₇₀LA₃₀/TB 10% or 20% (wt/wt) Relative to SRP Alone

	Formulation	PPD (mm)	Recession	BOP (%)
Study A	POE/TB 10%	- 0.67	+	- 50
	POE/TB 20%	- 0.50	+	- 33
Study B	POE/TB 20%	-0.75	+	- 57

Study A: SRP plus delivery system

Study B: SRP plus retraction cord plus delivery system

PPD: Periodontal Probing Depth

BOP: Bleeding on Probing

SRP: Scaling and Root Planing

TB: Tetracycline Base

Although it is clear that changes in PPD after only 10 days have to be interpreted with caution, one can say that the clinical parameter indicates an improvement on time in all treated sites. The formulations with 10% (wt/wt) TB loading showed better performance than those with 20% loading. This can be explained by the decrease in the adhesive properties of the polymeric system containing the drug.²⁷ Ten percent drug loading is sufficient to achieve concentrations in the GCF which are able to eliminate periodontal bacteria.

The reduced retention of the formulations in both sites (single-rooted or molar teeth with furcations) is probably due to the important bleeding observed after scaling and root planning. For this reason a second trial was conducted (study B), in which an attempt was made to stop the bleeding using a retraction cord before placing the formulations. Moreover, no difference was noticed between both types of sites, and no correlation between the site and the retention was observed.

Study B:

As for clinical study A, no complaints or signs of inflammation, irritation, or pus formation were noted, suggesting that the formulation was well tolerated.

The results indicate that 5 out of 6 patients included in the study presented at least 1 positive site for tetracycline during the 11-day trial (**Figure 8**). At the site level, 9 of 24 sites were positive for tetracycline at day 4; 6 of 24 sites were positive at day 7; and 2 of 24 sites were still positive at day 11. Tetracycline concentrations were generally well above the MIC (1 µg/mL - 2 µg/mL), except for 3 sites at day 7.

Figure 8. Study B. Tetracycline concentrations (mean ± SD) in positive sites after the placement of the delivery system.

The results of clinical study B showed marked improvement in the retention of the product in periodontal pockets compared with study A. Five of 6 patients presented positive sites for tetracycline in study B, whereas only 2 of 6 patients were positive in study A. Approximately 50% of treated sites were positive for tetracycline in study B, whereas only 15% of sites were positive in study A. In addition, 2 sites were positive for tetracycline at day 11 in study B compared with 0 at day 10 in study A. Clinical parameters seemed to indicate a more favorable response in study B compared with the study A for the same formulation (**Table 1**). Again, because of the short observation time, these findings need to be interpreted with caution.

We can conclude from these results that improvement of the application conditions (ie, stopping bleeding before placing the product) resulted in an increased retention of the formulation in the periodontal pocket. Under these improved conditions, tetracycline was released locally for a prolonged period, up to 11 days. However, the reasons why the formulation was not retained in all treated sites remain to be further investigated. No correlation could be established between retention of the product and the clinical situation or the application conditions.

CONCLUSION Preliminary results from 12 patients indicate that the local application of injectable bioerodible systems based on selfcatalyzed POEs containing tetracycline appear to be promising in the context of periodontal treatment. Therapeutic drug concentrations exceeding the MIC can be maintained in the gingival crevicular fluid for a period of at least 7 days. In addition to good tolerance, the formulations offer the advantages of ease of injection in the periodontal pocket and no need of product removal due to its biodegradability. Furthermore, by using an injectable formulation, it is possible to treat several sites using the same syringe.

Based on the results of both clinical trials, we can conclude that the POE injectable system has the potential to remain in the periodontal pocket for a prolonged period, up to 11 days. The retention of the formulation in the treated site is dependent on the conditions of application, as reduced bleeding significantly increased the retention of the product. The future directions envisioned would be (1) to improve the clinical protocol for the application of the new formulation by testing other hemostatic agents or by closing the pocket using adhesive components and (2) to evaluate the application and retention of the new formulation in multirooted teeth.

Long-term microbiological and clinical evaluations on a larger population with improved formulations are currently under investigation.

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