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Evaluation of a controlled-release compact containing tetracycline hydrochloride bonded to tooth for the treatment of periodontal disease

Augusta E.M. Collins [1], P.B. Deasy [1], Denise J. MacCarthy [2] and D.B. Shanley [2]

Departments of [1] Pharmaceutics and [2] Periodontology, Trinity College, University of Dublin, Dublin (Ireland)

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Summary

Compacts containing tetracycline hydrochloride and polyhydroxybutyric acid (PHB) were evaluated in vitro in simulated saliva pH 6.6 at 37 °C. Variation in compression pressure over the range 106–318 $kg \cdot cm^{-2}$ had negligible effect on drug release. Increase in drug loading from 30% to 60% caused a progressive increase in drug release. Decrease in average molecular weight of PHB or alteration to polylactic acid tended to reduce drug release. Increasing copolymerization of PHB with hydroxyvalerate tended to increase the initial drug release. Compacts containing 50% tetracycline hydrochloride physically dispersed in PHB as confirmed by differential scanning calorimetry, were evaluated in a panel of 12 patients suffering from gingivitis. The mean salivary level of drug produced was in the therapeutic range over the 10 day study period. The average plaque index, gingival index and pocket depth of the treated group showed desirable reduction in comparison to the control group, but the clinical improvement was not maintained when treatment was stopped. Examination of plaque samples by dark-field, phase-contrast and fluorescence microscopy with gram-staining confirmed a favourable alteration in microbial flora during treatment.

Introduction

Various pharmaceutical formulations containing antimicrobial agents are available for the topical treatment of oral diseases. They include lozenges, mouthwashes, aerosols and gels. However, none of the existing products have effective extended release, but are associated with an initial burst of active ingredient whose level rapidly declines to sub-therapeutic concentrations. Apart from compliance problems involved in frequent administration, such products are unsuitable for effective therapy overnight or for the long-term maintenance of drug levels of benefit in the treatment of periodontal disease. The latter condition includes gingivitis, acute necrotizing ulcerative gingivitis, periodontitis and periodontosis, the more severe forms of which may require systemic treatment with antibiotics, notably tetracycline, with the concomitant side-effects of prolonged oral therapy.

Various micro-organisms are considered to be involved in the initiation and progression of periodontal disease (Kelstrup and Theilade, 1974). It seems probable that specific groups of bacteria are associated with particular clinical forms of the disease (Marsh, 1980). Healthy periodontal conditions have simple bacterial populations that predominantly consist of non-motile coccoid and filamentous forms. Destructive periodontal dis-

ease is associated with a more complex microflora and increased numbers of spirochetes and motile rods are present in subgingival plaque at diseased sites (Listgarten and Hellden, 1978).

Polyhydroxybutyric/ polyhydroxyvalerate (PHB/PHV) polymers and copolymers are slowly biodegradable and are considered to have potential for use in controlled drug delivery systems as also have the more biodegradable polylactic acid/ polyglycolic acid (PLA/PGA) polymers and copolymers which do not compress as well. Compacts containing PHB were shown by Korsatko et al. (1983a and b) to prolong release of 7-hydroxyethyltheophylline in vitro and in vivo. Similar studies have also been carried out on PHB microparticles containing sulphamethizole by Brophy and Deasy (1985), who confirmed the suitability of this range of polymers for controlled release purposes.

TABLE 1

Compacts weighing 80 mg and 2 mm in thickness containing tetracycline hydrochloride (THCl) and a matrix (mx) of PHB, mol.wt. 1×10^5, 2×10^5, 5×10^5 (mx1, mx2, mx3), PLA (mx4) or PHB/PHV 17 mole % and 30 mole % (mx5, mx6) with both faces flat (types 1–12) or one face flat and one convex (type 13). Compacts 12 and 13 weigh 40 mg

Compact type	Composition (%) THCL : mx	Diameter (mm)	Compression force ($kg \cdot cm^{-2}$)
1	50 : 50 mx2	7.5	212
2	50 : 50 mx2	7.5	106
3	50 : 50 mx2	7.5	318
4	30 : 70 mx2	7.5	212
5	40 : 60 mx2	7.5	212
6	60 : 40 mx3	7.5	212
7	50 : 50 mx1	7.5	212
8	50 : 50 mx3	7.5	212
9	50 : 50 mx4	7.5	212
10	50 : 50 mx5	7.5	212
11	50 : 50 mx6	7.5	212
12	50 : 50 mx2	5	177
13	50 : 50 mx2	5	177

Materials and Methods

Materials

Acetic acid, ethylenediaminetetraacetic acid disodium salt (EDTA), sodium chloride (Riedel de Haen), acetonitrile (HPLC grade; Rathburn Chemicals), acridine orange, citric acid monohydrate, Cocktail T, dilute fuchsin, disodium hydrogen orthophosphate dodecahydrate, ethanol, iodine, Methyl violet, potassium nitrate (British Drug Houses), Concise composite (3M), helium (Irish Industrial Gases), immersion oil (Gurr), tetracycline hydrochloride (Sigma), polyhydroxybutyric acid (PHB; mol.wt. 1×10^5, 2×10^5, 5×10^5), polyhydroxybutyrate-co-polyhydroxyvalerate (PHB/PHV; 17 or 30 mole % hydroxyvalerate, mol.wt. 1.7×10^5 and 5×10^4 respectively; Marlborough Biopolymers), poly(DL = lactic acid) (PLA, mol.wt. 5×10^4, Polysciences) and glass-distilled water were used. All reagents were GPR unless otherwise specified.

Methods

Preparation of compacts

The components of each system were physically mixed together in the desired proportions using a mortar and pestle. A suitable quantity of the mixture was then loaded into a punch and die set with the required dimensions and a chosen compression force was applied using an infrared press. Various features of the compacts manufactured are given in Table 1.

Dissolution studies

Each compact was bonded onto an extracted molar tooth using Concise so that only the upper face was exposed prior to immersion into the dissolution medium at 37 °C, which was shaken twice daily. The absorbance of the dissolution medium was read at 353 nm using a UV spectrophotometer (Pye Unicam SP8-100) and the concentration of drug calculated by reference to a linear calibration curve constructed at the same pH. Absorbance values of potential interfering substances were negligible.

Gingivitis study

The clinical trial protocol was approved by the Dublin Dental Hospital Ethics Committee and the National Drugs Advisory Board. Twelve subjects,

aged 18–45 years, who had been clinically diagnosed as suffering from gingivitis were selected. Oral hygiene instruction was given to all on day 0. Six of the subjects had a type 13 compact bonded to an upper molar using Concise on day 7 and the remainder of the compact and bonding material was removed on day 17. Salivary samples were taken from the patients fitted with the compacts over the 10 day treatment period for measurement of drug levels.

Various clinical indices were evaluated for 8 teeth in each patient at the specified intervals. Plaque index after Silness and Loe (1964), which is a measure of oral hygiene, and gingival index after Loe and Silness (1963), which is a measure of gingival inflammation, were evaluated on a scale of 0–3 at 4 sections (buccal, lingual, mesial and distal) of each tooth. A score of 0 indicates no plaque or inflammation and a score of 3 indicates severe plaque or inflammation, respectively. The pocket depth, which is a measure of the severity of periodontal disease, was measured in mm at 6 locations around each tooth with a calibrated probe. Supragingival plaque samples were taken on the tip of a curette and suspended in 2 ml saline by vigorously agitating the tip in the saline. These samples were used to monitor the microbial flora.

Measurement of tetracycline salivary levels

Reversed-phase high-performance liquid chromatography (HPLC) was used to assay the drug content of the saliva samples using a modification of the method of Knox and Jurand (1979). A Shimadzu LC-5A liquid chromatograph equipped with a Shimadzu SPD-2A variable wavelength UV detector was used. Output from the detector was plotted and analysed using a Shimadzu C-R3A chromatopac integrator. The column used was a 10 cm Hypersil 5 ODS protected by a 5 cm precolumn packed with the same material. The mobile phase was a mixture of distilled water and acetonitrile (81.5 : 18.5 v/v) containing 3×10^{-3} M EDTA, 1.1×10^{-2} M KNO^3 and acetic acid to give a pH of 3.0. It was filtered through a 0.22 μm membrane filter (Matricel, Gelman) and degassed with helium prior to use.

The columns were preconditioned with the mobile phase until a stable baseline was obtained. A flow rate of 2 ml $\cdot$ min^{-1} was used and the eluent was monitored at 353 nm. In each case 20 μl of sample was injected. Several standards of tetracycline hydrochloride in mobile phase were run to establish injection to injection reproducibility. Blank saliva samples and saliva samples spiked with drug were run to ensure no interference would occur between the drug and other components of the saliva. Saliva samples were then injected to determine their level of tetracycline hydrochloride. Drug standards were injected daily between samples to obtain the required linear calibration plot of peak area versus concentration. The retention time of the drug was 4.21 min. The average saliva concentrations of lipids, fatty acids and proteins are considerably lower than those in plasma. Consequently the complex extraction procedures often used when assaying plasma samples are not generally required when monitoring the content of saliva samples.

Microscopy of plaque samples

(a)Dark-field and phase-contrast. To minimize loss of bacterial mobility, slides were prepared immediately after collection of plaque samples for examination within 1 h. Each plaque sample was coded so that the treatment received by the patient was unknown to the person doing the microscopy. The suspension of plaque in 2 ml of 0.85% sodium chloride solution was dispersed by aspirating and expelling the fluid through a disposable syringe fitted with a 23-gauge needle. One drop of suspension was placed on a glass slide and a coverslip applied. The preparation was examined by dark-field microscopy at a magnification of 1000 $\times$ under oil using a Nikon microscope. If the preparation was observed to be too dense, the sample was diluted with additional saline and another slide prepared. The bacteria from 3 fields, each containing about 100 organisms and selected at random, were classified as cocci, rods (motile and non-motile), fusiforms and spirochetes. The above procedure was repeated for phase-contrast microscopy using a Zeiss microscope.

(b) Gram-stain and fluorescence. One drop of a suitable dilution of the bacterial sample was spread on a glass slide, allowed to dry, heat-fixed in a bunsen flame and gram-stained. The organisms in 3 fields, each containing about 100 organisms and selected at random, were counted at a magnification of 1000 × under oil using a Zeiss microscope. For fluorescence studies one drop of a suitable dilution of the sample was spread on a glass slide, allowed to dry and heat-fixed as above. It was then stained using a few drops of Acridine orange solution 0.05%. After 5 min the acridine orange was washed off with water, the slide air-dried and 3 random fields each of about 100 organisms were counted at 1000 × under oil using a Nikon microscope.

Differential scanning calorimetry (DSC)

The thermal behaviour of tetracycline hydrochloride and PHB (mol.wt. 2×10^5) was examined alone and in combination, using a Mettler TC 10A TA Processor and an Epson FX-800 printer.

Results

In vitro dissolution studies

Fig. 1 shows the in vitro release of tetracycline hydrochloride from type 1 compacts which contain 50% drug and 50% PHB. The release shows the typical profile of a drug dispersed in an inert matrix (Deasy, 1984) with some 50% of the loading released in a pseudo-zero-order manner over the interval 8–9 days. The correlation coefficient for a linear fit was 0.9908, calculated using an Apple IIe computer, for the initial 10 day release which is significant at the P value of 0.001. The standard errors derived from 5 replicate determinations are shown as bars and indicate that the release profiles obtained were very consistent.

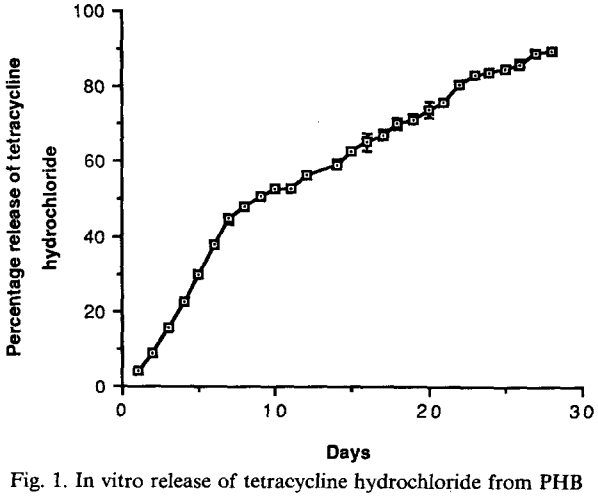

Fig. 1. In vitro release of tetracycline hydrochloride from PHB compacts (type 1) with 50% drug loading. The bar represents ±1 S.E.M.

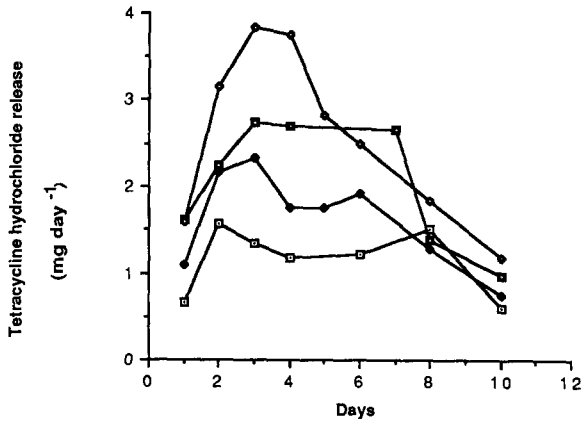

Fig. 2. In vitro release of tetracycline hydrochloride from PHB compacts (type 1, 4, 5, 6) with different drug loadings: ⊡, 30%; ◆, 40%; ◘, 50%; ◆, 60%.

Compact types 2 and 3 were used to study the influence of compaction pressure on drug release and the results obtained indicated negligible effect over the range studied. All products released 1.5–3 mg tetracycline hydrochloride per day in vitro for the first 8 days. The estimated salivary levels that would be produced in vivo are approximately 1.5–3 $\mu g \cdot ml^{-1}$ as the total daily secretion of saliva ranges from 500–1500 ml in adults. These levels would be well within the therapeutic range for the drug, as Addy et al. (1982) have reported that the minimum inhibitory concentration (MIC) of many oral pathogens is below 1 $\mu g \cdot ml^{-1}$.

The effect of drug loading was studied using compact types 4–6, which contained 30%, 40% and 60%, respectively, tetracycline hydrochloride in PHB. Fig. 2 shows the release with type 1 for comparison. The release tended to increase significantly with increasing drug loading as more drug

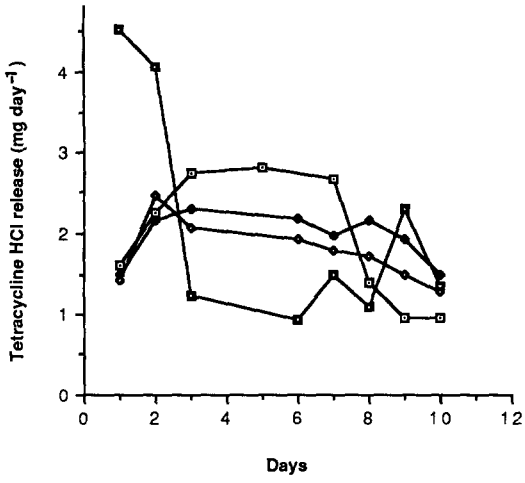

Fig. 3. In vitro release of tetracycline hydrochloride from PHB compacts (type 1, 7, 8) and from PLA compact (type 9) with different polymer molecular weights: PHB 100,000 (◆), 200,000 (□), 500,000 (■), PLA 50,000 (◆).

is exposed to the dissolution medium. Also a more porous matrix is progressively formed with higher loadings as more drug is leached out of the compact leading to a decrease in structural integrity of the device. This effect was observed for a 60% drug-loaded compact, when a large piece broke off after 6 days resulting in a large increase in drug release. As the 50% drug-loaded system tended to provide high but more constant release over a 10 day period and did not prematurely mechanically fail, this system was chosen for further study.

The effect of varying the molecular weight of PHB on the release of drug was investigated. Compact types 7 and 8 were produced containing PHB with a mol.wt. of 1×10^5 and 5×10^5, respectively. PLA was used as the matrix in type 9 compacts to see if a useful drug release profile would be obtained, particularly as PLA has been more thoroughly investigated for controlled release purposes and has regulatory approval. Fig. 3 shows the release of tetracycline hydrochloride from the various products including that using PHB, mol.wt. 2×10^5. The rate of release over the first 2 days was most rapid for the system containing the highest molecular weight of PHB. This is not unexpected as the higher the molecular weight of the polymer the more crystalline-like and brittle the polymer will be, leading to excessive fracture on compression with reduction in retardant effect. The release profiles from compacts 1 and 7 were similar but the product with the lower average molecular weight of polymer tended to release lower and less desirable quantities of drug over the 10 day period, presumably due to decreased fracture of the matrix with more effective entrapment of the drug. Likewise the release from PLA containing compacts tended to be undesirably low though similar to compacts 1 and 7. Apart from differences in chemical structure, this reduced release of drug can be explained by the more amorphous nature of DL-PLA which should deform better on compression than PHB, producing a compact with less pores through which drug molecules can diffuse. This effect would be partially counteracted by an increase in the rate of diffusion of drug molecules through the more amorphous polymer matrix, as it would be expected to offer a lower resistance to their passage than a more crystalline material. In addition, faster biodegradability of the PLA matrix would decrease its resistance to drug diffusion though this factor is unlikely to be significant over a time-span of only 10 days.

Compacts were made containing copolymers of PHB/PHV as the polymer matrix. The rate of release of tetracycline hydrochloride into simulated saliva from compacts containing 17 or 30 mole % PHV (types 10 or 11) was compared with the basic system over the initial 10 days (see Fig. 4). Over the first four days drug release from the system containing 30 mole % PHV was much faster. Nevertheless the initial rate of drug release was not maintained and neither system released constant quantities of drug daily for 10 days. Copolymers of PHB/PHV are more amorphous than the homopolymer PHB and would be expected to show less resistance to the diffusing drug molecules, resulting in a faster rate of drug release. However, copolymers should deform better on compression forming compacts with lower porosity as the matrix is more internally plasticized and more flexible than the homopolymer. Consequently diffusion of drug molecules through the polymer particles should be easier and diffusion around the polymer particles harder to accomplish

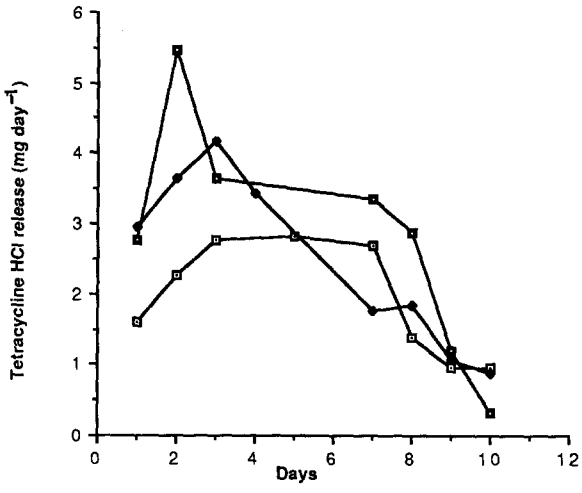

Fig. 4. In vitro release of tetracycline hydrochloride from PHB compacts (type 1) and from PHB/PHV compacts (types 10, 11): PHB matrix (⊡), 17 mole % PHV matrix (◆), 30 mole % PHV matrix ■).

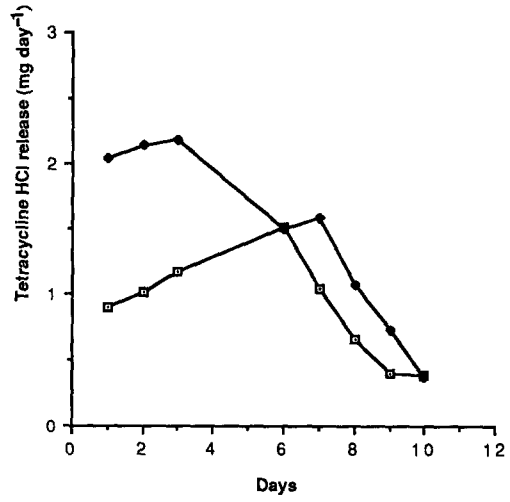

Fig. 5. In vitro release of tetracycline hydrochloride from PHB compacts (types 12, 13) with different surface geometry: exposed flat face (⊡), exposed convex face (◆).

in PHB/PHV copolymers than in PHB. A balance of these effects will determine the observed rate of drug release. It is likely that minimum porosity has been achieved in the system containing 17 mole % PHV and that its effect on reducing the release rate has been more than offset by easier diffusion through the polymer. Therefore, in the case of the product containing 30 mole % PHV a greater effect of increasing the ease of diffusion through the polymer particles would be achieved resulting in a very rapid release of drug from the polymer matrix.

It was decided to investigate the release profile for compacts 5 mm in diameter because devices 7.5 mm in diameter are quite large for attaching to the side-wall of a tooth and may be considered excessively bulky by the patient. Fig. 5 shows the in vitro rate of release of tetracycline hydrochloride from compact types 12 and 13. Both products were identical in diameter but differed in that the exposed face of type 12 was flat whereas type 13 was convex for improved patient comfort in the mouth. The faster release of drug from the 5 mm curved device is explained by the greater surface area exposed to the dissolution medium.

The release profile of compact type 12 was compared with that of the basic system, type 1. They differed only in the area exposed to the dissolution medium with diameters of 5.0 and 7.5 mm, respectively. Two cases were considered: in case I the amount of drug released per unit area was calculated on the assumption that the drug

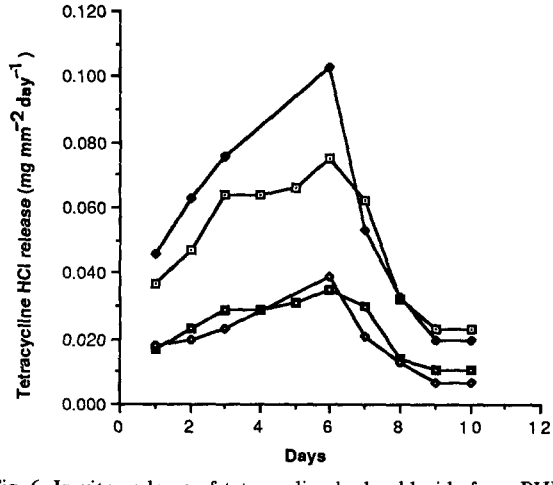

Fig. 6. In vitro release of tetracycline hydrochloride from PHB compacts (type 1, 12) with different dimensions assuming upper surface only or upper surface and side-walls exposed to the dissolution medium: type 1, upper surface only (⊡); type 12, upper surface only (◆); type 1, upper surface and side-walls (■); type 12, upper surface and side-walls (◆).

was being released from the upper face only whereas in case II drug release was considered to occur from the upper face and from the side-walls of the device (see Fig. 6). When the side-walls of the device are not taken into account, more drug seems to be released per unit area from the smaller compact. However, when the side-walls of the device are taken into account the drug is released at approximately the same rate from both products over the 10 day period. As there is no reason why the release rate of drug should differ other than if the side-wall of the compact was occluded in varying amounts by the mountant material, it is concluded that drug release from the side-walls is significant and contributes to the overall release of drug from the compact.

In vivo studies

Fig. 7 shows the mean in vivo saliva levels of tetracycline hydrochloride for 6 patients treated with a type 13 compact. The saliva levels produced in vivo by the compact are in the therapeutic range as $< 1\ \mu g \cdot ml^{-1}$ of tetracycline is effective against *Streptococci* and bacterioides organisms (Addy et al., 1982). After an initial increase in saliva level, the mean concentration decreased gradually until day 6 and then leveled off over the remainder of the 10 day period.

Fig. 8 shows the mean plaque index for the treatment and control groups during the study

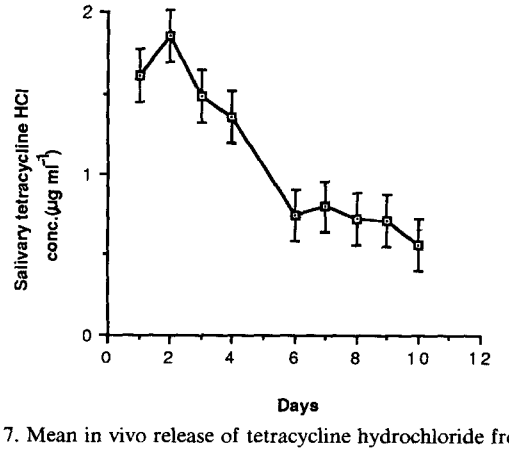

Fig. 7. Mean in vivo release of tetracycline hydrochloride from PHB compacts (type 13) in 6 humans. The bar represents ±1 S.E.M.

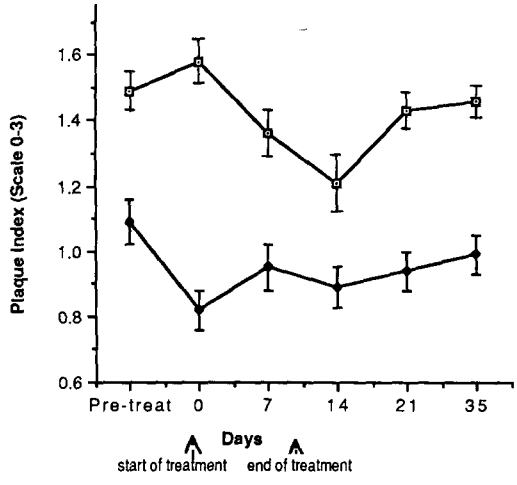

Fig. 8. Mean plaque index in patients with gingivitis: treatment group (□), control group (◆). The bar represents ±1 S.E.M.

period. Analysis of variance confirmed a significant treatment effect ($P = 0.0109$), visit effect ($P = 0.0308$) and treatment/visit interaction ($P = 0.0464$) were obtained for plaque index from day 0 to day 21. Thus the plaque index changed significantly with treatment and with visit in these patients. The treatment/visit interaction implies the pattern of change in both groups was different. The mean plaque index of the treatment group was 1.5–1.6 before treatment and it fell to approximately 1.2 after treatment indicating that tetracycline hydrochloride reduced the quantity of plaque present. However, the plaque index had risen to pre-treatment levels by the second follow-up visit at 35 days. The plaque index of the control group was lower indicating this group had lower levels of plaque than the treated group. This occurred despite the random allocation of patients to treatment or control groups.

The mean gingival index for the treatment and control groups during the study period are shown in Fig. 9. A significant visit effect ($P = 0.0375$) and treatment/visit interaction ($P = 0.0001$) were obtained for the gingival index from day 0 to day 21. Though the initial level of inflammation observed in the control group was lower as they had less plaque, the gingival index of the treatment group had decreased to the level seen in the control group after 7 days treatment with tetracycline

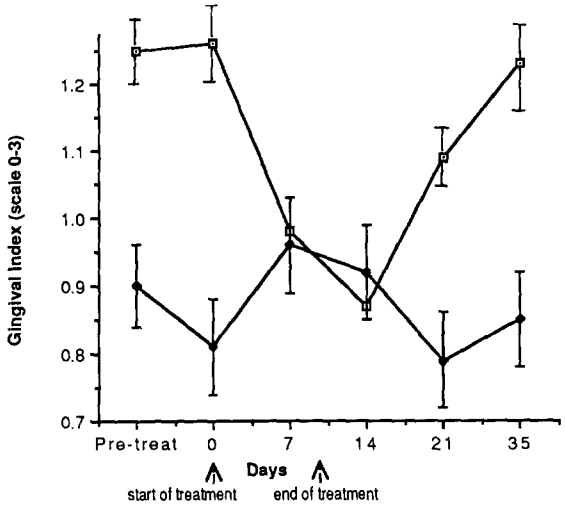

Fig. 9. Mean gingival index in patients with gingivitis: treatment group (⊡), control group (◆). The bar represents ±1 S.E.M.

hydrochloride. Thus the treatment brought about an improvement in the gingival condition from a case of moderate inflammation (gingival index scores 1.1–2.0) to mild inflammation (scores 0.1–1.0). Tetracycline hydrochloride reduces the quantity of plaque thereby decreasing the extent of gingival inflammation which is caused by bacterial toxins collecting around the gum margin. However, the reduction in gingival inflammation

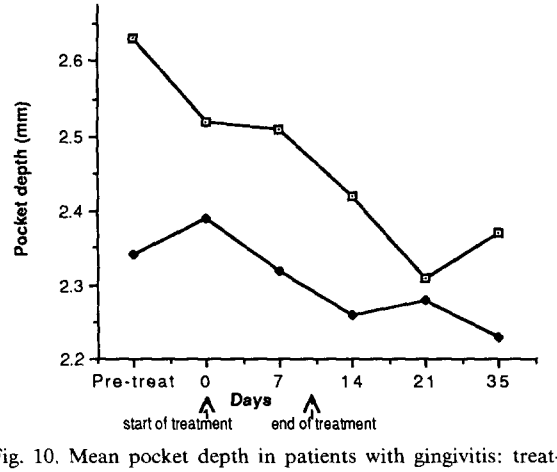

Fig. 10. Mean pocket depth in patients with gingivitis: treatment group (⊡), control group (◆).

was not maintained when the treatment was stopped as the gingival index had returned to pre-treatment levels by day 35.

Fig. 10 shows the mean pocket depth of the patients during the study. There was a significant treatment effect ($P = 0.0107$) and a significant visit effect ($P = 0.0032$) for pocket depth over the time period from day 0 to day 21. The mean pocket depth of the control group was less than that of the treatment group throughout. This was not unexpected as this group had less plaque and a lower degree of gingival inflammation than the treatment group. Some reduction in the mean pocket depth of the control group was also observed, presumably resulting from improved oral hygiene following prior instruction.

Microscopy studies on plaque samples

Various microscopic procedures were employed to examine plaque samples. Dark-field and phase-contrast microscopy were used to distinguish between motile and non-motile organisms. Gram-stained samples differentiated gram-positive and gram-negative organisms. Fluorescence was used because it provides a very clear view of the plaque sample with all the organisms stained particularly well.

The results obtained using the 4 techniques were compared. Fig. 11 shows the mean percentage cocci of total bacterial count using the 4 techniques in the 6 patients with gingivitis treated with type 13 compact. The results obtained using the different techniques correlate quite well. The proportion of cocci in the sample appeared higher when the sample was gram-stained than when other techniques were used, because the fusiforms and spirochetes did not stain well and were difficult to see. The lowest proportion of cocci was observed using fluorescence probably because all of the organisms were easily visible.

An increase in the mean percentage cocci of total bacterial count from 50% to 67% (approximately) occurred in treated patients (see Fig. 12). A significant visit effect was obtained for cocci over the first four visits ($P = 0.0171$) but there was neither a significant treatment effect nor a significant treatment/visit interaction. These results indicate that the proportion of cocci of total bacterial

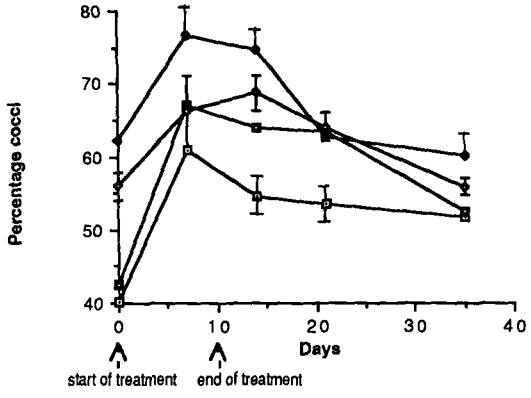

Fig. 11. Mean percentage cocci of total bacterial count in 6 patients with gingivitis treated with PHB compact (type 13) determined using different techniques: fluorescence (⊡), gram (◆), phase-contrast (■), dark-field (◈). The bar represents ±1 S.E.M.

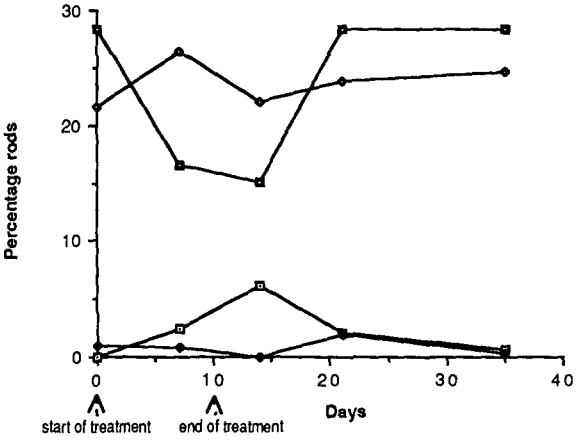

Fig. 13. Mean percentage gram-positive and gram-negative rods of total bacterial count in patients with gingivitis: G+ve rods treatment group (⊡), G+ve rods control group (◆), G−ve rods treatment group (■), G−ve rods control group (◈).

count changed with time in the gingivitis patients but that the pattern of the change was not statistically significant between the treatment and control groups. An increase in the proportion of cocci present is beneficial to the patient as coccoid cells predominate in plaque samples from non-diseased sites, whereas diseased sites generally have lower proportions of cocci present (Listgarten and Hellden, 1978). The increase in the proportion of cocci present during treatment with tetracycline hydrochloride was not maintained when the treatment

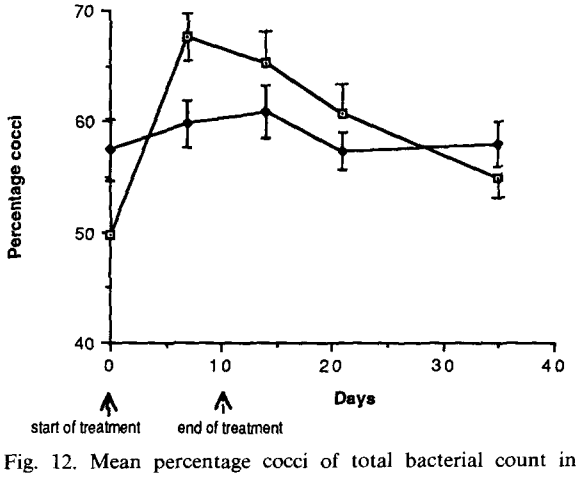

Fig. 12. Mean percentage cocci of total bacterial count in patients with gingivitis: treatment group (⊡), control group (◆). The bar represents ±1 S.E.M.

finished as no significant difference was obtained with respect to cocci by the second follow-up visit at day 35.

Fig. 13 shows that the increase in the proportion of gram-positive rods was counteracted by a larger decrease in the proportion of gram-negative rods resulting in an overall decrease in the mean percentage rods of bacterial count. Thus tetracycline hydrochloride was particularly effective against gram-negative rods which are considered important in the maintenance of periodontal inflammation (Slots, 1979). No significant change was observed in the proportion of motile rods present during treatment and they comprised less than 10% of the total bacterial count throughout the study period. A decrease in the proportion of motile organisms present is considered desirable as large numbers of motile bacteria have been associated with diseased conditions (Newman and Socransky, 1977).

The mean percentage fusiforms of total bacterial count decreased from 10% to 4.6% (approximately) in the gingivitis patients treated with tetracycline hydrochloride (see Fig. 14). The mean percentage fusiforms in these patients was 5–6% 10 days after the end of treatment (day 20) but they had increased to over 8% of the total bacterial

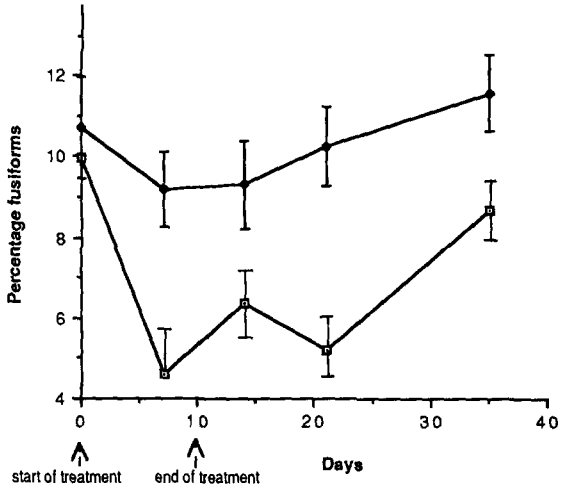

Fig. 14. Mean percentage fusiforms of total bacterial count in patients with gingivitis: treatment group (⊡), control group (◆). The bar represents ± 1 S.E.M.

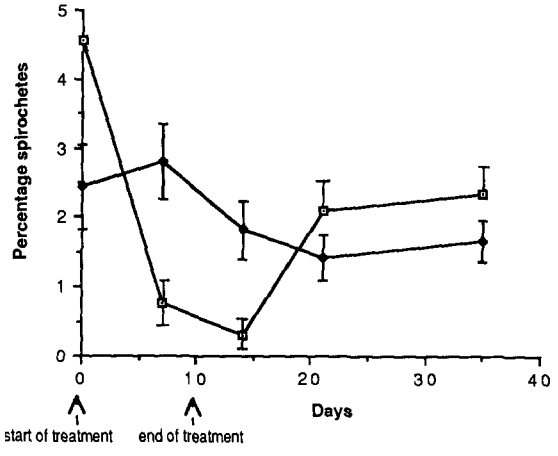

Fig. 15. Mean percentage spirochetes of total bacterial count in patients with gingivitis: treatment group (⊡), control group (◆). The bar represents ± 1 S.E.M.

count by day 35. A significant treatment effect ($P = 0.0197$) and a significant visit effect ($P = 0.0127$) were obtained for fusiforms. However, there was no treatment/visit effect indicating the pattern of change was similar in both groups. There was a statistically significant difference between the mean proportion of fusiforms in the treatment and control groups on day 35 ($P = 0.0495$). A reduction in the level of fusiforms in the oral flora of gingivitis patients is desirable as high levels of fusiforms are associated with diseased conditions.

The proportion of spirochetes in the supragingival plaque of the treated patients decreased from 4.6% to 0.4% (approximately) of the total bacterial count (see Fig. 15). Thus spirochetes were highly sensitive to local treatment with tetracycline hydrochloride. However, the percentage spirochetes of total bacterial count had increased to approximately 2% 10 days after the end of treatment, indicating that further therapy is required to maintain low levels of these microorganisms. No significant treatment effect or treatment/visit interaction was obtained though there was a visit effect ($P = 0.0516$). This result indicates that treatment cannot be significantly related to the change in the level of spirochetes. A significant difference between the treatment and control groups on day 35 was not observed.

Differential scanning calorimetry

Table 2 shows the thermal events of selected samples. The event for PHB at 174–176 °C corresponds to the crystalline melting temperature reported for PHB (Marchessault et al., 1981; Grassie et al., 1984). Mixtures of tetracycline hydrochloride and PHB showed similar thermal events to the individual components of drug and polymer. This result indicated that the drug was present in the crystalline state and therefore was dispersed rather than dissolved in the polymer matrix.

A conventional time$^{1/2}$ plot was drawn to see if the data would obey the square-root of time de-

TABLE 2

Thermal analysis of selected samples using differential scanning calorimetry

Sample	Thermal event (°C)
PHB	174–176 (endothermic)
Tetracycline HCl	220–221 (exothermic)
Tetracycline HCl/PHB (50:50)	174 (endothermic)/ 220 (exothermic)
Tetracycline HCl/PHB (25:75)	174 (endothermic)/ 221 (exothermic)

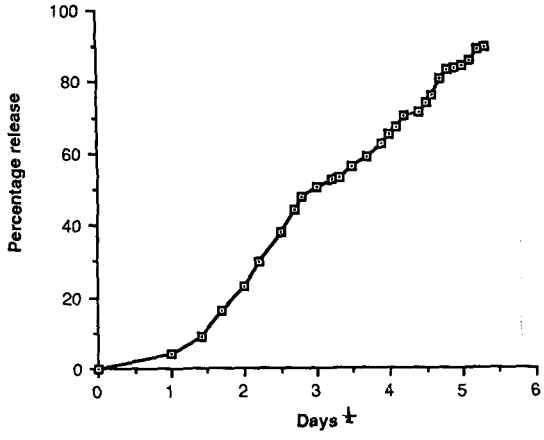

Fig. 16. In vitro release of tetracycline hydrochloride vs time$^{1/2}$ for PHB compact (type 1).

pendence predicted by the Higuchi (1963) model for drug release from inert matrix systems with dispersed drug particles. No erosion of the polymer was observed over the time course of in vitro studies. Fig. 16 shows the plot obtained for type 1 compacts. Apart from an initial lag and some tailing the plot was roughly linear having a correlation coefficient of 0.9913 which is significant at the P value of 0.001.

Discussion

Local delivery of drugs to the oral cavity has the advantage over systemic therapy of achieving higher concentrations at the intended site of action using less dosage with an associated reduction in side-effects. With the reduction in the incidence of dental caries in the population due to fluoridation, dentists will increasingly concern themselves with the treatment of sub-acute conditions such as gingivitis and with preventive care. The product discussed in this communication shows considerable potential for clinical use in such areas. As it requires the intervention of a dentist to mount and remove, it is likely to be well received by the profession, particularly as existing self-administered products lack efficacy because of short residence time in the mouth and poor patient acceptability. However, the tetracycline product will not be suitable for use in young children as the drug will stain permanent teeth when given to those under 8 years of age.

The tetracycline hydrochloride compact was relatively small and unobtrusive. No subject experienced any discomfort or irritancy from the device and the product was tasteless. PHB is a very suitable polymer for use in devices of this type because of its ease of compression and excellent ability to control drug release for long periods. In addition to the use of the device in the treatment of gingivitis, it may be also useful for treating many other oral conditions such as supragingival plaque, recurrent oral ulceration and "dry socket". Many other drugs could also be incorporated into the device such as cetylpyridinium chloride, metronidazole, miconazole, nystatin, betamethasone, hydrocortisone and triamcinolone. Further publications will describe some of these applications.

References

Addy, M., Rawle, L., Handley, R., Newman, H.N. and Coventry, J.F., The development and in vitro evaluation of acrylic strips and dialysis tubing for local drug delivery. *J. Periodontol.*, 53 (1982) 693–699.

Brophy, M.R. and Deasy, P.B., In vitro and in vivo studies on biodegradable polyester microparticles containing sulphamethazole. *Int. J. Pharm.*, 29 (1985) 223–231.

Deasy, P.B., *Microencapsulation and Related Drug Processes*, Dekker, New York, 1984, pp. 289–319.

Grassie, N., Murray, E.J. and Holmes, P.A., The thermal degradation of poly((D)-β-hydroxybutyric acid): Part 1. Identification and quantificative analysis of products. *Polymer Degrad. Stabil.*, 6 (1984) 47–61.

Higuchi, T., Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J. Pharm. Sci.*, 52 (1963) 1145–1149.

Kelstrup, J. and Theilade, E., Microbes and periodontal disease. *J. Clin. Periodontol.*, 1 (1974) 15–35.

Knox, J.H. and Jurand, J., Mechanism of reversed-phase separation of tetracyclines by high-performance liquid chromatography. *J. Chromatogr.*, 186 (1979) 763–782.

Korsatko, W., Wabnegg, B.B., Braunegg, G., Lafferty, R.M. and Strempfl, F., 1. Development of parenteral matrix tablets for long term application of pharmaceuticals. *Pharm. Ind.*, 45 (1983a) 525–527.

Korsatko, W., Wabnegg, B. and Tillian, H.M., 2. Development of parenteral matrix tablets for long term application of pharmaceuticals. *Pharm. Ind.*, 45 (1983b) 1004–1007.

Listgarten, M.A. and Hellden, L., Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. *J. Clin. Periodontol.*, 5 (1978) 115–132.

Loe, H. and Silness, J., Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol. Scand.*, 21 (1963) 533–551.

Marchessault, R.H., Coulombe, S., Morikawa, H., Okamura, K. and Revol, J.F., Solid state properties of poly-β-hydroxybutyrate and of its oligomers. *Can. J. Chem.*, 59 (1981) 38–44.

Marsh, P., Oral Microbiology, Nelson, Walton-on-Thames, 1980, pp. 1–88.

Newman, M.G. and Socransky, S.S., Predominant cultivable microbiota in periodontitis. *J. Periodontol. Res.*, 12 (1977) 120–128.

Silness, J. and Loe, H., Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol. Scand.*, 22 (1964) 121–135.

Slots, J., Subgingival microflora and periodontal disease. *J. Clin. Perio.* 6 (1979) 351–382.