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Use of Strips Containing Tetracycline Hydrochloride or Metronidazole for the Treatment of Advanced Periodontal Disease

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Abstract—Strips containing tetracycline hydrochloride or metronidazole 25% in polyhydroxybutyric acid as a biodegradable polymer matrix, showed sustained release in simulated gingival fluid pH 6·6 at 37°C. When evaluated in patients suffering from advanced periodontal disease, the greatest response to therapy was observed with tetracycline hydrochloride strips inserted into periodontal pockets at four-day intervals for 16 days, compared with an untreated control group. A reduction in plaque index, gingival index and pocket depth was observed. A favourable alteration occurred in the microbial flora of treated pockets with an increase in the proportion of cocci and decrease in gram-negative rods, fusiforms and spirochetes. Metronidazole strips or root-planing tended not to be as effective. The clinical improvement produced by each treatment was not maintained when treatment was terminated.

Periodontal disease is a general term for several conditions such as gingivitis, acute necrotizing gingivitis, periodontitis and periodontosis in which the supporting tissues of the teeth are attacked. The disease affects virtually the whole of the world's population and is a major source of tooth loss after the age of 25 years. Most current treatment procedures are directed towards an elimination of the total plaque mass subgingivally as well as supragingivally (Rosling et al 1983). They include oral hygiene instruction, scaling and root-planing. However, many studies have indicated that antimicrobial therapy can be useful in the treatment of such patients. Tetracyclines administered systemically have been used for many years in the treatment of all forms of periodontal disease (Ciancio 1976; Socransky 1977; Slots et al 1979). Metronidazole may be of use in cases of chronic progressive periodontitis where anaerobes are implicated as pathogens and it is the drug of choice in strictly anaerobic infections as it can eliminate the pathogens without disturbing the commensal aerobic flora of the mouth. Studies using metronidazole suggest that the reduction of obligate anaerobes in subgingival plaque, rather than the elimination of all bacteria, is of importance in the treatment of chronic periodontitis (Lindhe et al 1983).

Local treatment of periodontal disease with antibacterial agents has principally been by mouth rinses, lozenges and to a lesser extent by topical application in an adhesive carrier. Many workers have shown how the local delivery of such agents fails to reach the periodontal pocket which is the site of destructive periodontal disease (Bain & Strahan 1978; Pitcher et al 1980). In an attempt to improve subgingival drug delivery, local irrigation (Soh et al 1982) and the insertion of various types of sustained release device into the periodontal pocket have been investigated. Goodson et al (1979) used hollow fibres of cellulose acetate filled with a 20% solution of tetracycline hydrochloride which were pressed below the margin of the gingiva. Though improving the periodontal microflora, more than 95% of the drug was released within 1 h necessitating very frequent replacement. A monolithic fibre of polyethylene vinylacetate impregnated with tetracycline hydrochloride gave extended release (Goodson et al 1983) which could be maintained by weekly replacement of the product. Ethylcellulose strips containing 30% chlorhexidine inserted into periodontal pockets gave effective drug release locally over three days (Soskolne et al 1983). Acrylic strips containing 40% w/w chlorhexidine, metronidazole or tetracycline for insertion into pockets were reported by Addy et al (1982) to give marked improvement in the local microflora.

This paper describes the production and in-vitro/in-vivo evaluation of a new type of antimicrobial strip containing tetracycline hydrochloride or metronidazole in a biodegradable matrix of polyhydroxybutyric acid for the treatment of advanced periodontal disease.

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Materials and Methods

Chemicals

Acridine orange, citric acid monohydrate, dilute fuchsin, disodium hydrogen orthophosphate dodecahydrate, ethanol, iodine, methyl violet, potassium nitrate (British Drug Houses), glutaraldehyde (Taab laboratories), immersion oil (Gurr), metronidazole, tetracycline hydrochloride (Sigma), M-glue (Agar Scientific), polyhydroxybutyric acid-PHB, $\bar{M}_w$ 2×10^5 (Marlborough Biopolymers) and glass distilled water were used. All chemicals were GPR unless otherwise specified.

Preparation of strips

The components of a system were physically mixed in a mortar and pestle. Aliquots were compressed between flat-faced punches in a die using an infrared press. Various features of the strips produced are given in Table 1.

Dissolution studies

Each strip was placed in a stainless steel coarse mesh basket which was suspended in 100 mL of McIlvaine buffer pH 6·6

Table 1. Strips containing tetracycline hydrochloride (THCl) or metronidazole (M) as drug and PHB as matrix prepared at a compression force of 106 kg cm^{-2} in a 7·5 mm die fitted with flat-faced punches; each strip was 0·5 mm in thickness.

Strip type	Wt (mg)	Composition (%) Drug:Matrix
1	15	25 THCl:75
2	15	10 THCl:90
3	15	25 M:75

(simulated gingival fluid) at 37°C. The buffer was changed daily and its volume was sufficient to provide sink conditions for both drugs as tetracycline hydrochloride and metronidazole are soluble 0·1 g and 1·18 g, respectively in 100 mL of the buffer at 37°C and no compact contained more than 4 mg drug. The frequent change of dissolution medium was necessary because of the poor stability of tetracycline hydrochloride under the dissolution conditions (Knox & Jurand 1979). Release of drug occurred from both faces of a strip as this would occur in-vivo if it was placed in a periodontal pocket. The absorbance of the dissolution medium was read daily at 353 nm for tetracycline hydrochloride or 320 nm for metronidazole and the concentration of drug calculated by reference to the appropriate drug calibration curve constructed at the same pH.

Periodontitis study

The clinical trial protocol was approved by the Dublin Dental Hospital Ethics Committee and the National Drugs Advisory Board. At specified intervals, plaque index after Silness & Loe (1964), which is a measure of oral hygiene, and gingival index after Loe & Silness (1963), which is a measure of gingival inflammation, were determined on a scale of 0–3 at four locations at the three selected sites in each patient. 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 six sites for each pocket. Immediately before insertion and after removal of the strips, a subgingival plaque sample was obtained on the tip of a fine curette and suspended in saline. Supragingival plaque was first removed by polishing the tooth surface.

Microscopy of plaque samples

For dark-field and phase-contrast microscopy, in order to minimize loss of bacterial mobility, slides were prepared immediately after collection of plaque samples for examination within 1 h. Each sample was coded to prevent recognition of the patient treatment by the person doing the microscopy. The suspension of plaque in 2 mL of saline 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. Each preparation was examined at a magnification of × 1000 under oil using a Nikon microscope for dark-field or a Zeiss microscope for phase-contrast studies. If the preparation was observed to be too dense, the sample was diluted with additional saline and another slide prepared. The bacteria in three fields each containing about 100 organisms and selected at random were classified as cocci, rods, fusiforms or spirochetes.

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 or stained with acridine orange solution 0·05% for fluorescence microscopy. The organisms in three random fields each about 100 were counted at a magnification of × 1000.

Statistical analysis of data

Analysis of variance was used to interpret the data using the software package BMDP. The normal level of significance, $P=0·05$ was used throughout. The data for the study were analysed to see if the periodontal pockets showed a treatment effect, a visit effect and/or a treatment/visit interaction with respect to cocci, rods, fusiforms, spirochetes, plaque index, gingival index, pocket depth and attachment loss over the study period to day 16. The data were also analysed to see if there was a significant difference between the pockets on the follow-up visits with regard to the same variables. The "visit effect" refers to a significant difference in pockets with time (over a series of visits), whereas the "treatment effect" refers to a significant difference between pockets receiving different treatments.

Differential scanning calorimetry (DSC)

The thermal behaviour of tetracycline hydrochloride, metronidazole and PHB was examined alone and in combination using a Mettler DSC 20 standard cell equipped with a Mettler TC 10A TA Processor and an Epson FX-800 printer.

Results and Discussion

In-vitro dissolution studies

The in-vitro release profile of PHB strips containing tetracycline hydrochloride or metronidazole is shown in Fig. 1. Increasing loading of tetracycline resulted in increased drug release, whereas at a constant drug loading of 25% metroni-

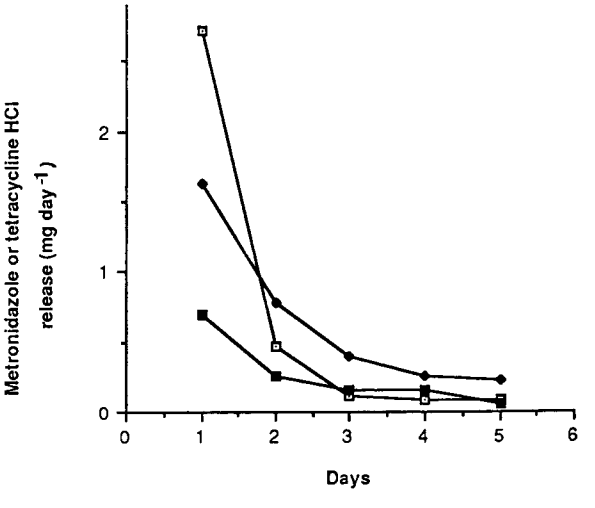

FIG. 1. In-vitro release of metronidazole or tetracycline hydrochloride from PHB strips: 25% metronidazole (□), 25% tetracycline HCl (◆), 10% tetracycline HCl (■).

dazole was released much faster. The quicker release of metronidazole was due to its greater solubility at the pH of the dissolution medium. The various strips remained intact over the 5 day dissolution period but progressively became fragile with loss of mechanical strength. Drug release from such strips is probably slower and more uniform in-vivo within a poorly perfused periodontal pocket as adequate sink conditions are less likely to exist.

In-vivo studies

Fig. 2 shows the mean plaque index for the treatment and control groups during the study period. Using analysis of variance, a significant visit effect ($P=0{\cdot}0003$) and a significant treatment effect ($P=0{\cdot}02$) were obtained for plaque index over the treatment period in the group of patients that received type 1 tetracycline hydrochloride containing strips. The mean plaque index of 1·4 approx. before treatment fell to 0·5 approx. after 16 days treatment with tetracycline hydrochloride, indicating a considerable reduction had occurred in the quantity of plaque present. However, when the drug treatment was stopped, a progressive increase in plaque index occurred. In contrast, the reduction in the plaque index for the root-planed and metronidazole (type 3 strips) treatments did not differ significantly from the control group. The decrease that occurred in the control group was probably caused by improved oral hygiene of the subjects as they all received instruction at the start of the study.

The mean gingival index for the various treatment and control groups is shown in Fig. 3. A significant visit effect ($P=0{\cdot}0006$) was obtained for the tetracycline hydrochloride group of patients over the treatment period. The metronidazole group had a significant treatment effect ($P=0{\cdot}04$). A reduction in the gingival index from 1·2 to 0·6 and from 1·3 to 0·9 approx. was obtained with 16 days treatment with tetracycline hydrochloride and metronidazole, respectively. Thus both treatments brought about an improvement in the gingival condition from a case of moderate inflammation (gingival index scores 1·1–2·0) to mild inflammation (gingival index scores 0·1–1·0). However, the reduction in gingival inflammation was not maintained when the treatment stopped, as a significant treatment effect was not obtained for either group of patients on day 70.

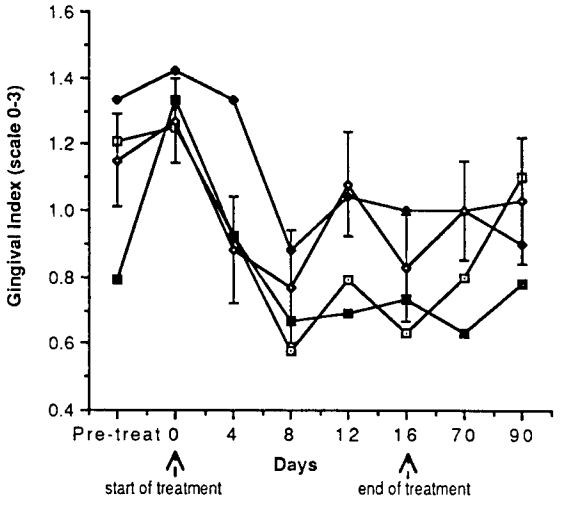

FIG. 3. Mean gingival index for patients treated for periodontal disease: tetracycline HCl (⊡), metronidazole (◆), root-plane (■), control (◇). The bar represents ± 1 standard error.

Fig. 4 shows the mean pocket depth for the various treatment and control groups. A significant visit effect ($P=0{\cdot}0005$) was obtained for pocket depth over the treatment period for the group of patients that received tetracycline hydrochloride strips. The mean pocket depth of the pockets treated with tetracycline hydrochloride was 5·3 mm approx, which was deeper initially than the other groups (all 4·5 mm approx) but the depth was reduced over the treatment period.

Microscopy studies

Supragingival plaque around the pocket was removed before taking each sample as it could distort the true composition of the bacterial flora in the immediate vicinity of the affected tissue. Dark-field and phase-contrast microscopy were used

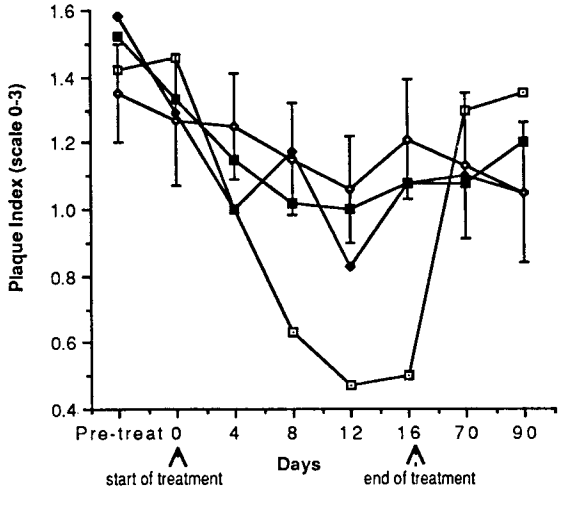

FIG. 2. Mean plaque index for patients treated for periodontal disease: tetracycline HCl (⊡), metronidazole (◆), root-plane (■), control (◇). The bar represents ± 1 standard error.

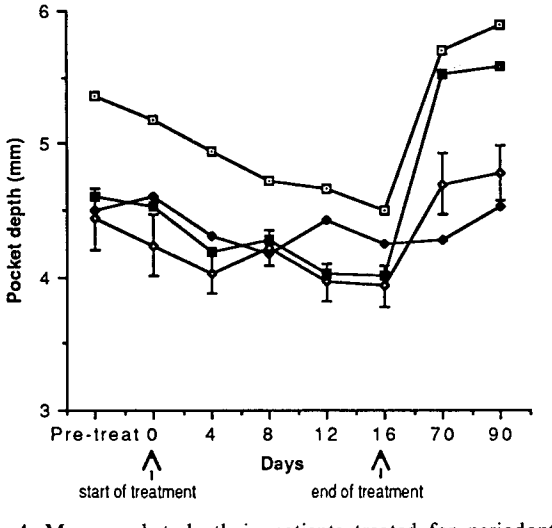

FIG. 4. Mean pocket depth in patients treated for periodontal disease: tetracycline HCl (⊡), metronidazole (◆), root-plane (■), control (◇). The bar represents ± 1 standard error.

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 clear view of the plaque sample with all the organisms staining particularly well.

The results obtained using the four techniques were compared. Fig. 5A shows the mean percentage cocci of total bacterial count using the four techniques in the six pockets treated with type 3 metronidazole strips. The results obtained using the different techniques correlate quite well. The proportion of cocci in the sample appeared higher when it was gram-stained than when the other techniques were used, because the fusiforms and spirochetes did not stain well and were difficult to see. The lowest percentage of cocci was obtained using fluorescence because all of the organisms were easily visible.

An increase in the mean percentage cocci of total bacterial count from 51 to 78% approx. occurred in the pockets treated with type 1 tetracycline hydrochloride strips for 16 days (see Fig. 5B). This increase was larger than the increase in the mean percentage cocci in the pockets that were treated with metronidazole or root-planed. A significant visit effect was obtained for cocci over the first five visits in both groups of patients receiving either tetracycline hydrochloride strips ($P=0{\cdot}04$) and those treated with metronidazole strips ($P=0{\cdot}0003$). A significant treatment effect ($P=0{\cdot}04$) was also obtained for the metronidazole group. An increase in the proportion of cocci present is desirable as such organisms predominate in plaque samples taken from non-diseased sites, whereas diseased sites generally have lower proportions of cocci present (Listgarten & Hellden 1978). Unlike the metronidazole treatment, the increase in the mean percentage cocci in the pockets treated with tetracycline hydrochloride was not maintained when the treatment finished as observed on the first follow-up visit at day 70. No significant difference existed between the test pockets in either group of patients by day 90. Tetracycline hydrochloride treatment was observed to markedly increase the gram-positive cocci but caused a reduction of the gram-negative cocci within the mean percentage observed. Metronidazole treatment caused a similar but less marked effect. No significant change was observed in the proportion of motile cocci present in any of the treatment groups and they comprised less than 10% of the total bacterial count throughout the study period.

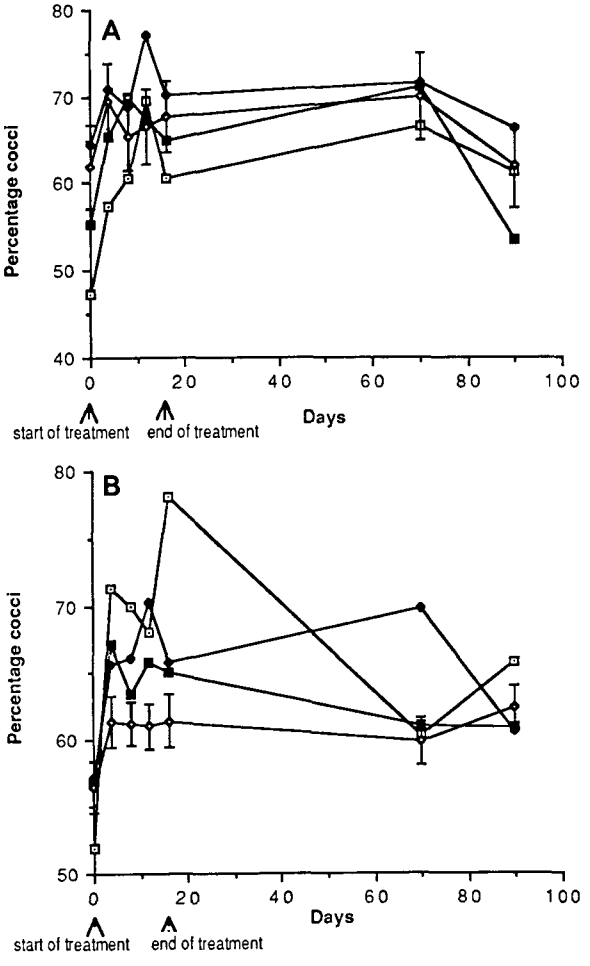

FIG. 5.A. Mean percentage cocci of total bacterial count determined using different techniques in six patients treated for periodontal disease with type 3 metronidazole strips: fluorescence (□), gram (◆), phase-contrast (■), dark-field (◇). The bar represents ± 1 standard error.

B. Mean percentage cocci of total bacterial count in patients treated for periodontal disease: tetracycline HCl (□), metronidazole (◆), root-plane (■), control (◇). The bar represents ± 1 standard error.

Fig. 6A shows the mean percentage rods of total bacterial count for the various treatment procedures. The mean percentage rods in the pockets treated with tetracycline hydrochloride decreased from 31% to 20% of total bacterial count during treatment but increased when the treatment stopped, returning to pre-treatment levels by the first follow-up visit at day 70. A significant visit effect ($P=0{\cdot}02$) was obtained for rods in the tetracycline hydrochloride group during the treatment period. This group showed an increase in the proportion of gram-positive rods which was counteracted by an even larger decrease in the portion of gram-negative rods, confirming the effectiveness of tetracycline against the latter class of rods. A reduction from 22% to 16% was also observed in the proportion of gram-negative rods present in pockets during treatment with metronidazole. Reduction in gram-negative rods is important as such microorganisms are considered to be involved in the maintenance of periodontal inflammation (Slots 1979).

Fig. 6B shows the mean percentage fusiforms of total bacterial count in the periodontal pockets involved in the study. A significant treatment effect ($P=0{\cdot}02$), visit effect ($P=0{\cdot}02$) and treatment/visit interaction ($P=0{\cdot}05$) were obtained for fusiforms over the treatment period in the group of patients that received treatment with tetracycline hydrochloride strips. The treatment/visit interaction implies that the pattern of change in the pockets treated with tetracycline hydrochloride, root-planed and the controls was different. A reduction in the proportion of fusiforms from 6·2% to 1% approx. of total bacterial count was achieved in the pockets treated with tetracycline hydrochloride but they had increased to over 4% of the total bacterial count by the first follow-up visit at day 70. No significant change in the proportion of fusiforms was obtained for the subgingival plaque samples from the pockets receiving metronidazole using analysis of variance. This is not surprising as metronidazole is specific for anaerobes and has little activity against

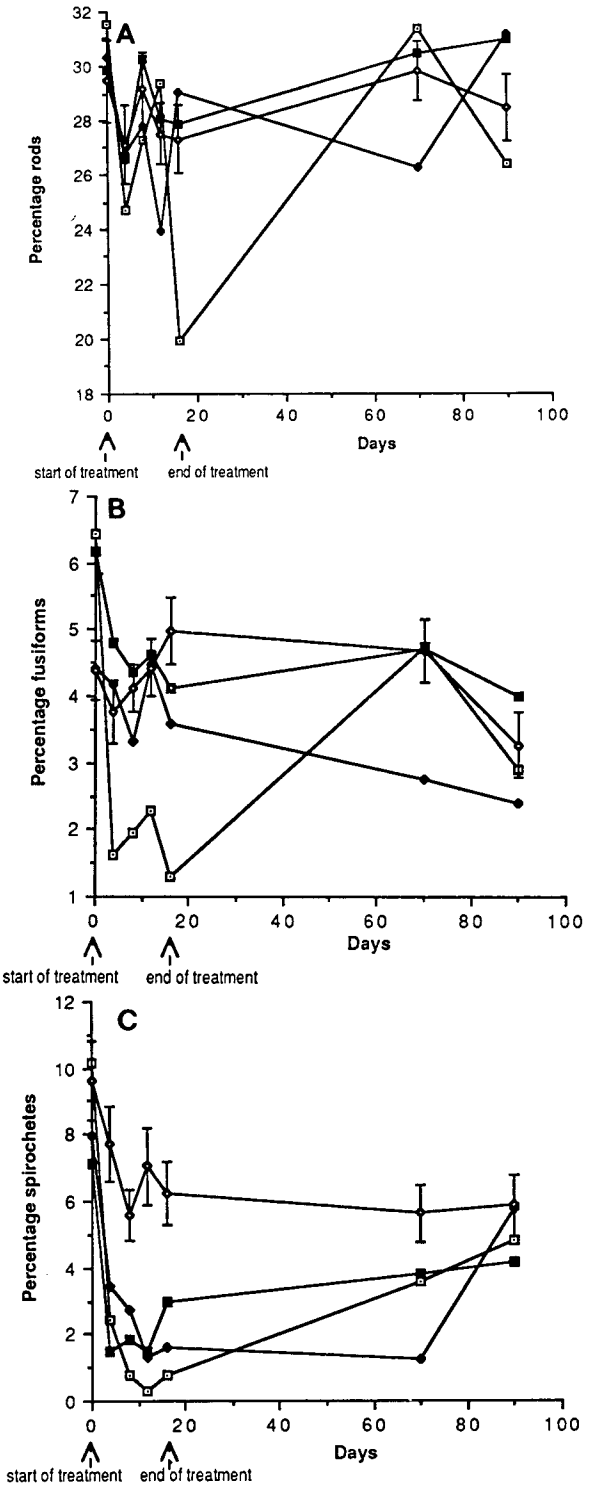

FIG. 6A. Mean percentage rods of total bacterial count in patients treated for periodontal disease: tetracycline HCl (□), metronidazole (◆), rootplane (■), control (◇). The bar represents ±1 standard error.

B. Mean percentage fusiforms of total bacterial count in patients treated for periodontal disease: tetracycline HCl (□), metronidazole (◆), root-plane (■), control (◇). The bar represents ±1 standard error.

C. Mean percentage spirochetes of total bacterial count in patients treated for periodontal disease: tetracycline HCl (□), metronidazole (◆), root-plane (■), control (◇). The bar represents ±1 standard error.

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)
Metronidazole	161 (exothermic)
Metronidazole/PHB (25:75)	161 (exothermic)/175 (endothermic)

aerobes. The proportion of motile fusiforms present in all treatment groups was low, comprising less than 2% of the total bacterial count throughout the study period. The gram-stained samples indicated that the fusiforms were gram-negative. A reduction in the level of fusiforms is desirable as high levels are associated with diseased states.

The proportion of spirochetes in the subgingival plaque from the pockets that were treated with tetracycline hydrochloride or metronidazole and those that were root-planed decreased considerably during the treatment period, when compared with the control pockets (see Fig. 6C). The greatest decrease was seen in the pockets treated with tetracycline hydrochloride. Significant treatment effects ($P=0{\cdot}02$, tetracycline hydrochloride group; $P=0{\cdot}01$, metronidazole group) with significant visit effects ($P=0{\cdot}02$, tetracycline hydrochloride group; $P=0{\cdot}02$, metronidazole group) were observed. The low spirochete levels were maintained longest in the pockets treated with metronidazole (until day 70) and shortest in pockets that were root-planed (until day 12).

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 by Marchessault et al (1981); Grassie et al (1984). Mixtures of tetracycline hydrochloride or metronidazole with PHB showed similar thermal events to the individual components of drug and polymer. These results indicated that both drugs were present in the crystalline state in the products used in the in-vivo studies and therefore were dispersed rather than dissolved in the polymer matrix.

The greatest response to therapy was observed in pockets treated with tetracycline strips. Frequent use of the strips should be acceptable to the patient as no subject complained of any irritation, discomfort or unpleasant taste and the device was not dislodged in any case. However it would be necessary to investigate the composition of the microbial flora of the pockets at regular intervals to see if long-term use of the product led to the development of resistant strains. The product is likely to be well received by the dental profession, who will be actively involved in its use, because it can produce much higher levels of drug at the site of interest with no side-effect in comparison to conventional oral therapy for prolonged periods.

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