In vitro elution of ofloxacin from a bioabsorbable polymer

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A drawback with antibiotic-loaded polymethylmethacrylate (PMMA) beads is the second surgical procedure that may be required to remove the beads. We explored alternatives for the local delivery of antimicrobials and studied the release characteristics of ofloxacin (10 mg) when incorporated into either 30 or 50 mg beads of two different molar ratios of a bioabsorbable lactide: glycolide polymer. An elution method was employed over a 60-day period at 37 °C. After 60 days, about half of the ofloxacin had been released from the composites containing 50 mg and 30 mg of the 85:15 polymer, whereas nearly all of the ofloxacin had been released from both sizes of the 50:50 polymer composites.

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Delivering an effective antimicrobial at sufficiently high concentrations to the area of infection in combination with surgery is a recognized treatment for bone infection, and it is usually performed with the use of polymethylmethacrylate (PMMA) bone cement beads (Buchholz and Engelbrecht 1970, Buchholz et al. 1981) in combination with standard treatments for bone infection. The success rate varies from 40% to 90% (Carlsson et al. 1978, Wilson et al. 1978, Hunter 1979, Jupiter et al. 1981, Miley et al. 1982, Turner et al. 1982, Cherney and Amstutz 1983, Canner et al. 1984, Carlsson et al. 1985, Wroblewski 1986). One problem is that a second surgical procedure may be required to remove the beads.

An ideal delivery system should provide 1) adequate antimicrobial concentrations at the target site, 2) slow, constant release of antimicrobials over prolonged periods, and be 3) bioabsorbable. In an attempt to find such a delivery system for antimicrobials, we prepared several bioabsorbable antibiotic composites of ofloxacin and a lactide:glycolide polymer and determined the in vitro elution characteristics of this antibacterial.

Material and methods

Ofloxacin-polymer composites

Two different ofloxacin-impregnated polymer composite sizes (30 and 50 mg of polymer) were fabricated. The molar ratios of the polymer components (Medisorb Technologies International LP, Cincinnati, OH) are 85:15 and 50:50 DL-lactide:glycolide, respectively. The molecular weights of these two polymer mixtures are approximately 110–160 and 70–110, respectively. Ofloxacin (R W Johnson Pharmaceutical Research Institute, Raritan, NJ) analytical grade powder was kindly provided by the manufacturer.

The larger composite was made with 50 mg polymer and the smaller contained 30 mg, each composite contained 10 mg ofloxacin. Sufficient quantities of the polymer and ofloxacin powders to prepare a single composite bead were weighed. The composite components were then placed in a glass tube and melted by heating to 50 °C on a heating block for 3–4 minutes. The mixture was stirred while heating. The melted mixture was then placed in a 1.5 mL microcentrifuge tube (Baxter Diagnostics Inc., McGaw Park, IL) and extruded into a circular cone-like bead with a sterile stainless steel rod in an expeditious manner. All procedures were conducted using an aseptic technique. The diameter of the bottom of the

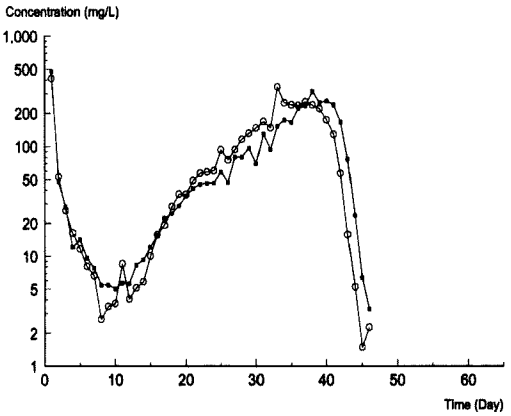

Figure 1. Ofloxacin release from 50 mg (■) and 30 mg (○) 50:50 polymer composites.

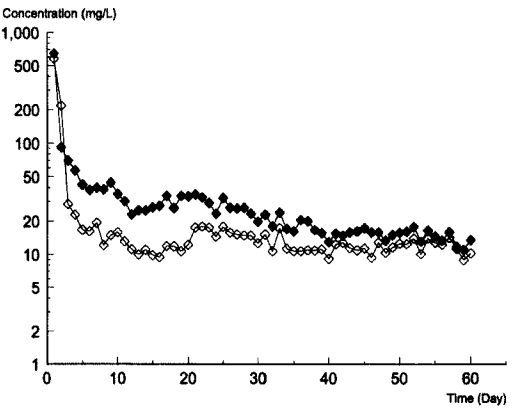

Figure 2. Ofloxacin release from 50 mg (◇) and 30 mg (◆) 85:15 polymer composites.

50 mg composite was 3.5 mm and the height was 4.6 mm, while the 30 mg composite was 3 mm by 4 mm, respectively.

In vitro elution study

An elution method was employed to determine the release characteristics of ofloxacin from the polymer composites. A phosphate buffer, 0.15 mol/L (pH 7.1), was used as the dissolution medium. 4 groups of samples were studied simultaneously. The groups were:
1) 50 mg of 85:15 polymer plus ofloxacin 10 mg,
2) 30 mg of 85:15 polymer plus ofloxacin 10 mg,
3) 50 mg of 50:50 polymer plus ofloxacin 10 mg,
4) 30 mg of 50:50 polymer plus ofloxacin 10 mg.

Two antibiotic composites were placed in each glass test tube (10 tubes per group) with a volume of 4 mL phosphate buffer. All tubes were incubated at 37 °C. After every 24-hour period, the dissolution medium was collected, and stored at –70 °C until the time of ofloxacin analysis. Fresh phosphate buffer (4 mL) was then added for the next 24-hour period and this procedure was repeated for 60 consecutive days.

Ofloxacin heat stability

Since it is necessary to heat the antibiotic-polymer composite to 50 °C for 3–4 minutes during the bead preparation, the chemical stability of ofloxacin had to be confirmed. A sufficient quantity of ofloxacin powder was weighed and diluted in 0.15 mol/L phosphate buffer to yield final concentrations of 2.5, 5, 10, 20, 40, 50, 80, and 100 mg/L. Aliquots of these standard concentrations were then placed in a heating block at a constant temperature of 50 °C for 60 minutes. Samples were obtained from these aliquots prior to heating, then at 5, 10, 20, and 60 minutes of heating. All samples were obtained in duplicate and were immediately stored at –70 °C until the time of ofloxacin concentration analysis.

Ofloxacin concentration

The ofloxacin concentrations in buffer for both the elution and stability studies were determined by an ultraviolet spectrophotometric method at a wavelength of 340 nm. Prior to the assay of the unknown samples, ofloxacin standard concentrations of 2.5, 5, 10, 30, and 50 mg/L were prepared in the buffer solution previously used for both experiments. This assay was linear over the concentration range of 2.5–50 mg/L. The interday and intraday coefficients of variation were less than 4% and 0.25%, respectively, for both the high- and low-quality control samples. All samples were assayed in duplicate and sample dilutions were performed, as required, to bring the unknown concentrations into the range of the assay standard curve. The Student's t-test was used.

Results

The release of ofloxacin from groups 1 and 2 (85:15 polymer) into the dissolution solution showed a common pattern in that the release was greatest in the first days after adding the phosphate buffer and slowly decreased thereafter (Figure 1). Ofloxacin was released from the 50 mg 85:15 composites to a greater extent than from the smaller 30 mg composite. After 60 days of elution, approximately 46% and 61% of the ofloxacin still remained in the 50 mg and 30 mg composites, respectively. The elution rate of the 50 mg composite from day 5 to day 60 was 2.83 (SD 1.05)% per 5-day period, whereas that of the 30 mg product was 1.60 (SD 0.27)% ($p < 0.001$).

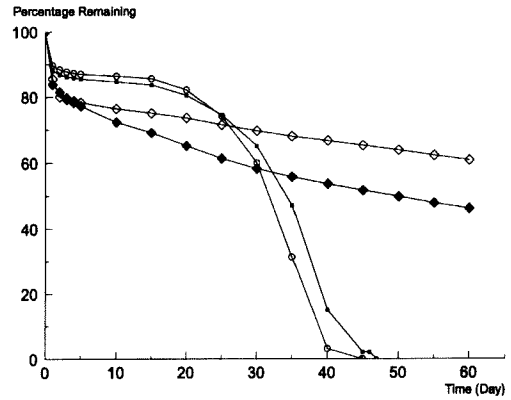

Figure 3. Percentage of ofloxacin remaining in composites over a 60-day period. 50 mg (■) and 30 mg (○) 50:50 polymer composites and 50 mg (◇) and 30 mg (◆) 85:15 polymer composites.

The 50:50 polymer composites revealed a different releasing pattern from that of the 85:15 composites. The release of ofloxacin decreased during the first 10 days from 500 to 4 mg/L per day, then increased very quickly to 250 mg/L by day 40. Unlike the 85:15 composites, the 50:50 composites became very sof, then came an apparent rapid release of the remaining ofloxacin (Figure 2). At day 46, the concentration of ofloxacin in the elution fluid was below the lowest level of assay detection. This result suggests that nearly all of the ofloxacin had been released from the 50:50 polymer composites over a 7-week period. The percentage of ofloxacin remaining per unit time for each of the four composites studied over the 60-day period is displayed in Figure 3.

During the ofloxacin stability study, the ofloxacin was found to be unchanged and negligible degradation occurred at 50 °C over a 60-minute period.

Discussion

Recently, several investigators have reported on the development and use of biodegradable and bioabsorbable antibiotic composites (Ikada et al. 1985, Visscher et al. 1985, Wei et al. 1991). We selected ofloxacin for our study because of its broad spectrum of antimicrobial activity for both Gram-positive and Gram-negative bacteria. In our study, the 50:50 DL-lactide/glycolide copolymer had the fastest degradation rate among the polymer materials used, since negligible concentrations could be determined in the elution fluid after 46 days. In contrast, the 85:15 DL-lactide/glycolide polymer composites appear to elute drug for approximately 150 days, a value quite similar to that which has been previously observed in vivo (Visscher et al. 1985). Our study suggests that both the volume ratio of polymer:ofloxacin and the ratio of lactide:glycolide can alter the release characteristics of the incorporated antimicrobial. The 85:15 DL-lactide/glycolide composites showed a similar releasing pattern, however, the differences observed in the rate of release can be attributed to the volume ratio of polymer:ofloxacin. Despite the substantial differences in the rate of ofloxacin release, both groups appear to provide concentrations that would exceed the minimum bactericidal concentration of many pathogens isolated from bone infections for a period of at least 60 days. The observed releasing pattern of ofloxacin in our study was similar to that of gentamicin-impregnated PMMA beads reported by other investigators (Holm et al. 1976, Wahlig et al. 1978, Wahlig and Dingeldein 1980, Hoff et al. 1981, Wahlig 1981, Flick et al. 1987).

As a result of the elution characteristics of ofloxacin reported here, it appears that the 50:50 DL-lactide/glycolide polymer may be a good choice for a drug delivery matrix for surgical prophylaxis where it may be necessary to achieve very high local concentrations of antibiotic early in the postoperative period whereas the 85:15 polymer composites may be more suitable for the treatment of bone infection where high local concentrations are required for prolonged periods of time.

Since the fabrication process of the absorbable composites requires that the antibiotic and polymer mixture be heated to approximately 50 °C not all antibiotics are suitable because of heat instability. Therefore, prior to the routine incorporation of antibiotics into this composite system, adequate data must be available concerning the effect of heating on the degradation of the chosen product.

In clinical use, the release of antibiotics will depend on at least two factors: the surface area of the chosen composite and the diffusion gradient of the affected tissue/fluid surrounding the composites. The in vitro results reported in this study suggest that this type of polymer composite is a viable option for the local delivery of antibiotics. However, additional in vivo studies of this absorbable ofloxacin-impregnated composite should be performed.

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