ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1999, p. 714–716 Vol. 43, No. 3
0066-4804/99/$04.00+0

Lactic Acid Polymers as Biodegradable Carriers of Fluoroquinolones: An In Vitro Study

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Received 5 May 1998/Returned for modification 3 November 1998/Accepted 26 December 1998

A biodegradable polymer of DL-dilactide that facilitates release of ciprofloxacin or pefloxacin at levels exceeding MICs for the causative microorganisms of chronic osteomyelitis is described. Duration and peak of release were found to depend on the molecular weight of the polymer. Its characteristics make it promising for treating chronic bone infections.

The elevated antibiotic tissue levels necessary for treating chronic bone infections might be achieved by a local delivery system. If the system is biodegradable, its removal after completion of release could be avoided (1). A new biodegradable system with incorporated ciprofloxacin and pefloxacin is described.

(Presented in part at the 33rd Annual Meeting of the Infectious Diseases Society of America, September 1995, San Francisco, Calif.; abstract 158.)

Polymers with molecular masses averaging 2, 26, and 100 kDa, each having a melting point of 90°C, were prepared. L-Lactic acid (for the production of the 2-kDa polymer) and crystalline DL-dilactide (Aldrich, Devente, The Netherlands [for the production of the 26- and 100-kDa polymers]) were diluted in acidic ethyl ester (Mallinckrodt, Chesterfield, Missouri) by continuous stirring under hot conditions to ensure the purity of the substance from any admixtures and were then transferred to an oven and kept at 50°C for 18 to 20 h. After drying, they were mixed with stannous-2-ethyl-hexanoate 95% [$Sn(Oct)_2$; Sigma Co., St. Louis, Mo.) diluted in toluene (Sigma Co.), which was used as a catalyst, and put into air-free spherical tubes (Mallinckrodt). They were then immersed for 20 h in an oil-bath kept at 140°C in which the $Sn(Oct)_2$ concentration (vol/vol) was 0.07 to 0.08%. The polymerization product was diluted in dichloromethane (Mallinckrodt) and was allowed to precipitate following the addition of methanol (Lab Scan Techline, Dublin, Ireland). The sediment was dried in an oven at 50°C for 20 h. The ability to obtain the prepared polymers each time was based on the catalyst concentration and the vacuum of the spherical tubes, whereas selection of the 26-kDa polymer as an intermediate-molecular-weight polymer was based on its stability compared with various prepared polymers.

One hundred milligrams of ciprofloxacin (Bayer, Leverkusen, Germany) or pefloxacin (Rhône-Poulenc Pharmaceutical Co., Vitry-sur-Seine, France) was added to the liquified polymer at a ratio of 1:10. After adequate stirring, the mixtures were poured into tubes and left to cool and solidify at 37°C. The mixture packed at the bottom of each test tube was a slab with a height of 4.4 mm and a diameter of 16 mm and thus had a free surface area of 200 mm^2, a total volume of 880 mm^3, and a free cross-sectional area of 150 mm^2. Tubes were sterilized by UV light, and they were closed firmly to prevent any contamination. Five tubes were prepared per quinolone.

One milliliter of sterile Mueller-Hinton broth (Oxoid Ltd., London, United Kingdom) was added to the free surface of the mixture, which was then left to incubate at 35°C. At 24-h intervals the entire amount of broth was removed in a sterile manner and kept refrigerated at −70°C until determination of the quinolone levels. It was then replaced after thorough washing of the interior of the test tube by sterile pyrogen-free water; this process was continued until decomposition of the mixture was apparent. Quinolone levels (expressed in micrograms per milliliter) were determined by a microbiological agar well diffusion assay on Mueller-Hinton agar (Oxoid Ltd.) with the application of *Escherichia coli* 14 (ICB 40-04) as an indicator strain. Drug levels were estimated by a standard curve created with known quinolone concentrations and plotted on semilogarithmic paper (2).

On each day of sampling the mean or the median value for the five test tubes was determined (11). The mean values (± standard deviation [SD]) of ciprofloxacin or pefloxacin release were calculated (Tables 1 and 2). Comparisons were performed by Student's *t* test or the Mann-Whitney U test ($P < 0.05$) (9).

Quinolone levels released in vitro by all polymers ranged from 100- to 1,000-fold of their MICs for the common causative pathogens of chronic osteomyelitis (5, 6). The 2-, 26-, and 100-kDa polymers were degraded in vitro within 56, 103, and 350 days, respectively. As a consequence the two latter polymers might be considered the most appropriate candidates for therapy for chronic osteomyelitis, which usually lasts 3 to 6 months (6, 8, 10). However, the enhanced diffusion gradient observed in vivo might result in more rapid drug release than that in vitro, which could shorten the period needed for the eradication of a chronic bone infection; this phenomenon was found for the duration of pefloxacin release by a 2-kDa polymer implanted intramedullary in the shaft of a rabbit tibia for only 33 days (4).

Quinolone release by the 2-kDa polymer reached its peak by day 20 and then steadily decreased until the degradation of the

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TABLE 1. In vitro release of ciprofloxacin by the three lactic acid polymers

Day(s)	Ciprofloxacin levels (μg/ml) (mean ± SD) achieved by polymer with molecular mass of:		
	2 kDa[a,b]	26 kDa[a,c]	100 kDa[a,d]
1	13	46‡	1,760**
2	100‡	97§*	440†‡†
3–4	30 ± 0‡	74.5 ± 3.5§†	274 ± 93.3‡§§
5–10	64.2 ± 12.4†	88.3 ± 22.7§‡	156.2 ± 31.2‡†*
11–13	165.3 ± 68.9†	72.3 ± 13.1§*	152.8 ± 20.7§†§
14–15	500 ± 141.4‡	84.5 ± 3.5§‡	134.4 ± 6.8§†§
16	1,500‡	46§†	125§§‡
17	650‡	86§‡	130§§‡
18	350‡	58§‡	112§§‡
19–20	217.5 ± 10.6§	54.5 ± 14.8§†	90.6 ± 18.1§§‡
21–24	176.3 ± 10.3†	40.5 ± 4.4§*	103.8 ± 10.9§**
25–28	89.1 ± 7.7*	86.5 ± 43.2§*	78 ± 5.2†§§
29–32	70.5 ± 2.5†	230.3 ± 44.5†*	77.8 ± 7.3§*§
33–36	53.4 ± 2.9*	261.3 ± 43.7§*	86.4 ± 20.8§*‡
37–45	28.6 ± 3.2*	235 ± 31.3§*	72.5 ± 12.6§**
46–51	13.3 ± 1.4*	403.3 ± 258.4§*	58.6 ± 6.6‡‡*
52–85		358.8 ± 75.9§	57.5 ± 11.9§*
86–97		200.5 ± 23.9*	56 ± 13.5§*
98–240			56.3 ± 17.8§
241–350			28.3 ± 4.7‡
Total amount (mg) of released ciprofloxacin	8.55	23.13	21.13

[a] Statistical differences are indicated as follows: *, $P < 0.001$; †, $P < 0.01$; ‡, $P < 0.05$; §, not significant.
[b] Symbols refer to comparisons with the corresponding values for release on the previous day(s).
[c] Except for the value for day 1, for which the symbol refers to comparison with the corresponding value for the 2-kDa polymer, the first symbol refers to comparison with the corresponding value for the previous day(s); the second symbol, if given, refers to comparison with the corresponding value for the 2-kDa polymer.
[d] Except for the value for day 1, for which the first and second symbols refer to comparisons with the corresponding values for the 26- and 2-kDa polymers, respectively, the first symbol refers to comparison with release on the previous day(s), the second symbol, if given, refers to comparison with the corresponding value for the 26-kDa polymer, and the third symbol, if given, refers to comparison with the corresponding value for the 2-kDa polymer.

polymer. The 26-kDa polymer produced a later peak of drug release (by day 32 for ciprofloxacin and by day 47 for pefloxacin). The peak of release by the 100-kDa polymer was found to span the first two days, when the levels were extremely high, exceeding 2,000 μg/ml; thereafter, a step-wise decrease over time was observed. Both quinolones were released at higher levels by the 100-kDa polymer compared to the 26-kDa one, until day 30, after which the release by the 26-kDa polymer was

TABLE 2. In vitro release of pefloxacin by the three lactic acid polymers

Day(s)	Pefloxacin levels (μg/ml) (mean ± SD) achieved by polymer with molecular mass of:		
	2 kDa[a,b]	26 kDa[a,c]	100 kDa[a,d]
1	40	395*	2,164**
2	96.2‡	203**	1,872§**
3–7	152.4 ± 31.6‡	37 ± 14.1**	1,307.6 ± 168†**
8–12	470.3 ± 149.7†	33 ± 9.1§*	778.6 ± 67.6†*§
13–20	642.9 ± 178.3§	158.8 ± 35.3**	553.5 ± 43.8**§
21–30	209.1 ± 71.5*	244 ± 25.9*§	371.7 ± 52.8***
31–35	107.8 ± 12.3†	376 ± 53.7**	307 ± 18.9‡‡*
36–40	82.1 ± 9.2†	815 ± 232.9†*	327 ± 72.6§†*
41–47	50.2 ± 9.9*	1,191 ± 255.7‡*	263.4 ± 41.9§**
48–56	29.6 ± 3.6*	793.4 ± 81**	284.6 ± 50.8§**
57–73		610.3 ± 139‡	398.6 ± 86‡‡
74–82		403.3 ± 50.2‡	312 ± 0.37§§
83–102		260.1 ± 36.7*	279 ± 27.8§§
103–144			255.6 ± 25.6§
145–210			154.6 ± 24.3*
211–295			54.7 ± 24.4*
Total amount (mg) of released pefloxacin	12.05	45.29	70.94

[a] Statistical differences are indicated as follows: *, $P < 0.001$; †, $P < 0.01$; ‡, $P < 0.05$; §, not significant.
[b] Symbols refer to comparisons with the corresponding values for release on the previous day(s).
[c] Except for the value for day 1, for which the symbol refers to comparison with the corresponding value for the 2-kDa polymer, the first symbol refers to comparison with the corresponding value for the previous day; the second symbol, if given, refers to comparison with the corresponding value for the 2-kDa polymer.
[d] Except for the value for day 1, for which the first and second symbols refer to comparisons with the 2- and 26-kDa polymers, respectively, the first symbol refers to comparison with release on the previous day(s), the second symbol, if given, refers to comparison with the corresponding value for the 26-kDa polymer, and the third symbol, if given, refers to comparison with the corresponding value for the 2-kDa polymer.

superior. Ciprofloxacin levels produced by the 100-kDa polymer were in general higher than those produced by the 2-kDa polymer, while release by the 26-kDa polymer was superior to that by the 2-kDa one until day 29, after which release by the latter was demonstrated to be superior (Table 1). In contrast, the release of pefloxacin by the 2-kDa polymer was inferior to those by both the other polymers on the majority of days (Table 2).

The concept of the development of a biodegradable polymer as a carrier for local antibiotic delivery is widely described in the literature that has appeared over the last several years, and the major applied systems are polylactide/polyglycolide copolymers (6, 12, 13). In the animal models the applied antimicrobials were gentamicin, kanamycin, and polymyxin B (7, 13, 15). The critical factors influencing the efficiency of these systems are the levels of the antimicrobial locally achieved and the depth of drug diffusion (15). Fluoroquinolone levels released by our model were higher than those of ciprofloxacin eluted by polymethylmethacrylate cement (3) and also higher than the levels of ciprofloxacin released by fibrin clots around the implant (14).

Our study describes a novel biodegradable system with future potential as an in vivo carrier of quinolones permitting high levels of drug release for an extended period as verified by preliminary animal studies (4), demonstrating the in vivo relevance of the presented model.

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