Controlled release systems based on poly(lactic acid). An *in vitro* and *in vivo* study

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A new biodegradable delivery system based on poly(lactic acid) has been formulated, with potential applications in sustained antibiotic release against bone infection. The *in vitro* release of a new quinolone (pefloxacin) from low molecular weight poly(D,L-lactic acid) ($Mw = 2 \times 10^3$) lasted for 56 d whereas the *in vivo* delivery lasted 33 d. In both cases, the release rate is controlled by the drug diffusion and the polymer degradation, which seems to be the predominant factor. For the release experiments, discs were prepared from poly (D,L-lactide) ($Mw = 2 \times 10^4$) with drug loadings of 2% and 10% w/w. It was concluded that pefloxacin concentration remains higher than the Minimum Inhibitory Concentration (MIC) against the major causative bacteria of bone infection. The results indicate that the two different types of poly(lactic acid) can be used effectively in an implantable antibiotic release system.

1. Introduction

Poly(lactic acid) (PLA) has been extensively investigated for the controlled release of drugs, biodegradable surgical sutures and implants for the fixation of bone fractures, since it displays several advantages over conventional systems used in therapeutics. When a biodegradable polymer is used as a carrier in an implantable drug delivery system there is no need for surgical retrieval. The biodegradable polymers achieving the most success at this time are those prepared from lactic and glycolic acid. They combine a number of desirable properties, the most important being that their degradation rate can be controlled over a wide range by changing their molecular weight. So far PLA has been used as a drug carrier in the form of microspheres, cylinders, microcapsules etc [1–3].

PLA degrades to its monomer, lactic acid, which is a normal metabolite of the human body. The degradation of PLA proceeds essentially via hydrolysis of ester groups which takes place heterogeneously, and shows higher rates in the central part of the specimen than at the surface. This rate strongly depends on the morphological structure, the dimensions of the biodegradable implant as well as on the reaction conditions. The hydrolysis is autocatalyzed by carboxylic groups (i.e. the hydrolysis rate increases exponentially with time. The presence of residual monomer, low molecular weight compounds and oligomers greatly accelerates the degradation rate because their presence tends to increase chain flexibility and polymer hydrophilicity, and provides carboxylic groups for the autocatalytic reaction [4, 5].

Poly(D,L-lactide) (PDLLA) is an amorphous polymer with a glass transition temperature of 58 °C, and a wide range of melting temperatures, depending on its molecular weight. It can be synthesized by several methods. The preferred route for the production of high molecular weight PLA is ring-opening bulk polymerization of the lactide. Various metallic, organometallic, inorganic, and organic zinc and tin compounds have been used as initiators [6, 7]. Tetraphenyl tin, stannous chloride and stannous octoate have been found to be the most effective. Among these, a preferred initiator is stannous octoate because of its acceptance by the FDA as a food additive [8, 9].

In this work, we investigated the correlation between the *in vitro* and *in vivo* release of pefloxacin from D,L-Lactic acid oligomer as well as the drug release from two different molecular weights at 37 °C. The aim of this study was to record the drug delivery over a long period of time in order to explore the design parameters of an implantable drug delivery system with the appropriate features.

2. Experimental

2.1. Materials and methods

D,L-lactic acid in the form of an aqueous solution (90% w/w) was supplied by Sigma and D,L-lactide was kindly donated by Boehringer Ingelheim. D,L-lactide was recrystallized from ethyl acetate just before use. Stannous octoate (Sigma) was used as a catalyst without further purification. Pefloxacin mesylate dihydrate with 67% purity was purchased from Lab Royer Bellon (Rhone Poulenc).

2.2. Polymer synthesis

The PLA (viscosity-average molecular weight 2000) used in this work was synthesized directly from D,L-lactic acid by conventional condensation polymerization. The monomer was heated until the temperature reached 100 °C. Refluxing was continued slowly for 8 h and then xylene was added (to control rate of reflux). Then the temperature was increased to 140 °C for 6–8 h approximately.

Poly(D,L-lactide), with a viscosity–average molecular weight of 20000, was prepared by ring–opening bulk polymerization of D,L-lactide, which was previously recrystallized from ethyl acetate and dried at 50 °C for 18 h approximately. The monomer was put in a 100 ml Erlenmeyer flask and the initiator (0.07% w/w of lactide) was added in the form of a 0.5% w/v solution in toluene. The reaction vessel was closed and immersed into a thermostatically controlled oil bath at 140 °C for 20 h. The product was dissolved in dichloromethane and precipitated in methanol [8, 9].

The viscosity-average molecular weight was determined in chloroform with an Ubbelhode viscometer thermostatically controlled at 25 °C. Calculations were made using the Mark-Houwink equation [10]:

$$[n] = K\ \mathrm{Mv}^a$$

where: $K = 2.21 \times 10^4$ and $a = 0.77$.

Pefloxacin was incorporated into the melt of the PLA2000 at 90 °C. The pure drug content of each mixture was 10%. After mixing, the melted substance was poured into test tubes with a cross section of 150 mm^2, so that slabs of 1 g were prepared. One ml of nutrient broth was then added in every test tube and the specimens were placed in an incubator (37 °C). The nutrient broth, above the free surface of its sample, was removed every 24 h and replaced by a new solution of the same volume. The obtained samples of nutrient broth were stored in deep freeze (− 70 °C) until the pefloxacin concentration levels were determined with the microbiological method of diffusion in agar.

Poly(D,L-lactide) was melted at 90 °C and mixed with pefloxacin at concentrations of 2% and 10% (w/w) respectively. The mixture was compression molded at 5 tons for 1 h and shaped into discs 10 mm in diameter and 1.2 mm in height and were stored in an oven at 37 °C after immersion in 200 ml of buffer solution (pH = 7.4).

2.3. *In vitro* study

The drug levels in the *in vitro* study were determined by the method of diffusion in agar, whereas in the case of poly(lactide) pefloxacin concentration was measured spectrophotometrically using a Perkin-Elmer spectrophotometer at 271 nm.

In order to evaluate the swelling behavior of two different types of poly(lactic acid), PLA (Mw = 2000) and PLA (Mw = 20 000), discs 10 mm in diameter and 1.2 mm in height were prepared by compression molding at 5 tons for 1 h and these were immersed in 200 ml of phosphate buffer pH = 7.4. The specimens were placed in an oven at 37 °C. At hourly intervals the pre-weighed tablets were removed, gently wiped to remove the surface water and re–weighed. The degree of swelling was calculated using the following equation:

$$\text{Degree of swelling } (\%) = (Ws - Wd)/Wd \times 100$$

where Ws is the swollen weight of the matrix and Wd is the final dry weight of the same matrix at immersion time (t) in the buffer.

2.4. *In vivo* study

Pieces of 500 mg PLA2000 containing 10% pefloxacin were placed intramedullary in the shaft of the tibia in 36 rabbits divided into 12 groups. The animals were sacrificed at 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 and 33 days after implantation. Samples were taken from blood, from the tibial cortex around the area where the lactic acid oligomer was placed (four samples were obtained at intervals of 1 cm^3) from the surrounding tissues (muscles, skin), and finally from cancellous bone. The pefloxacin concentration levels were determined with the microbiological method of diffusion in agar.

3. Results and discussion

3.1. *In vitro* study

The study of the kinetics of drug release from biodegradable polymers is quite complicated since drug diffusion through the matrix and erosion of the matrix proceed simultaneously. The type of drug, the shape of matrix, its loading and many other factors determine the mechanism as well.

The release of pefloxacin from PLA(2000) matrix in terms of its content in the nutrient broth is plotted as o function of the log(time) in Fig. 1. The log vs log plot for the release process was selected since this expression seems to better describe the phenomenon compared to the plot M_t vs time. A linear increase of the log of drug concentration with the log of time is evident for the first 16 days. Then a sharp decrease is observed leading to very low concentrations of the drug after about 55 days. This is probably due to the fact that degradation needs a few days to start and diffusion is very slow in this stage.

The swelling of a tablet prepared from PLA2000 in terms of buffer uptake is illustrated in Fig. 2. It is obvious that 120 h (5 days) after immersion (of the sample) the disintegration of the matrix starts. It should be noted that we cannot compare the behavior of PLA2000 used in the

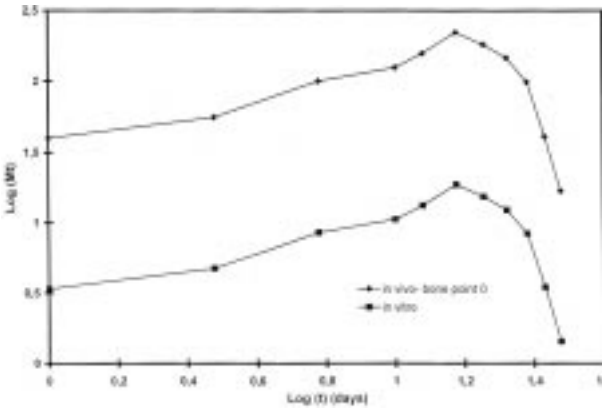

Figure 1 Pefloxacin concentration in the broth as a function of time.

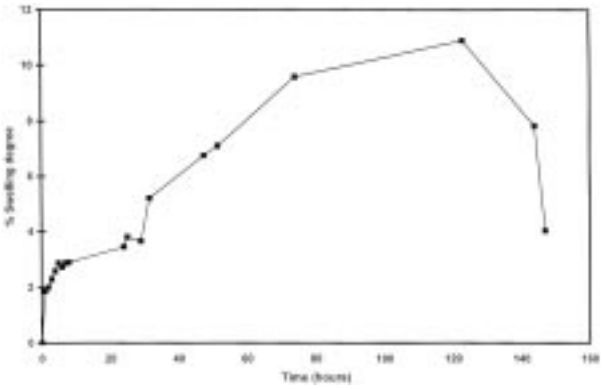

Figure 2 Degree of swelling for PLA 2000 samples as a function of time.

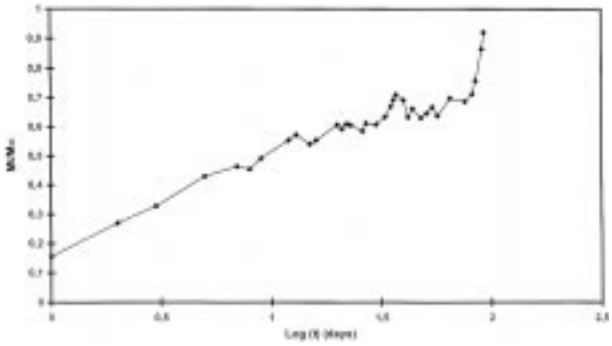

Figure 4 Release of pefloxacin from PLA discs (10% loading)–M_t/M_∞ versus log(t) [days].

in vitro release study with the one used in the swelling test since in the first case, only the top surface of the sample is in contact with the buffer solution.

The fraction of pefloxacin released from PLA 20000 (2%) and (10%) versus log of time is plotted in Figs 3 and 4, respectively. Release takes place for more than two months and, at the 70th day, 66–68% of the total drug content is delivered. The concentration of pefloxacin from PLA 20 000 (10%) increases constantly and reaches an equilibrium after the first 35 days, whereas in the case of PLA (2%) an initial burst effect is observed. In the case of PLA 20 000 (10%) Fick's law seems to apply for the first few days, giving an essentially linear curve. For further release, e.g during the first nine days, the plot of M_t/M_∞ versus the square root of time is no longer linear, as the regression coefficient drops to 0.9319 (Fig. 5).

It is obvious that the release from PLA (20 000) (2%) and PLA (20 000) (10%) is a two stage process. An initial rapid release rate is observed for 15 days, which reaches an equilibrium and finally 50 days after the immersion a sharp increase of the release rate is observed suggesting the acceleration of the matrix degradation. Once placed in an aqueous medium PLA absorbs water and hydrolytic cleavage of the ester bonds starts. The bulk degradation of PLA proceeds heterogeneously and goes more rapidly in the central part of the specimens than at the surface. At the beginning, degradation is faster at the surface than in the core as reported elsewhere [10]. Degradation products such as monomer and lactic acid oligomer are formed at the surface as well as in the core, but those localized near the surface can be dissolved more easily in the medium than those located inside the core. Consequently, the concentration of carboxylic end–groups increases in the center and therefore catalyzes ester degradation, resulting in a surface–center segregation. Eventually, as the molecular weight of the matrix decreases, water penetration is facilitated. Consequently, drug release through the channels or pores of the specimen is enhanced. The degree of swelling for PLA 20 000 versus time is plotted in Fig. 6 and it is obvious that the water absorption is a two stage process. During the first 50 days water absorption increased slowly and continuously. Between 50 and 60 days an acceleration occurred in the buffer uptake, corresponding to the beginning of the degradation of the sample. The sample made of PLA (20 000) shows higher equilibrium swelling which corresponds to about 110% increase in weight whereas in the case of PLA (2000) only an 11% increase in weight is observed. In fact, the disintegration takes a few days to start in the case of PLA (2000), so the gain from buffer uptake does not outweigh the weight loss which occurs due to matrix degradation. The difference in behavior observed between PLA (2000) and PLA (20 000) during the swelling test is attributed to the large difference in molecular weight.

3.2. *In vivo* study

Mean pefloxacin levels determined at various spots are listed in Table I. Pefloxacin level is extremely high one day after the implantation in the tibial cortex (point 0)

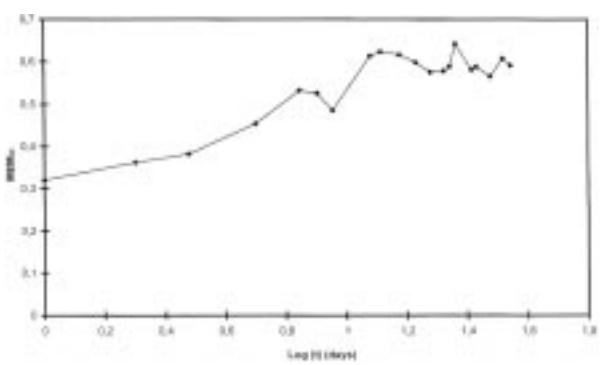

Figure 3 Release of pefloxacin from PLA discs (2% loading)–M_t/M_∞ versus log(t) [days].

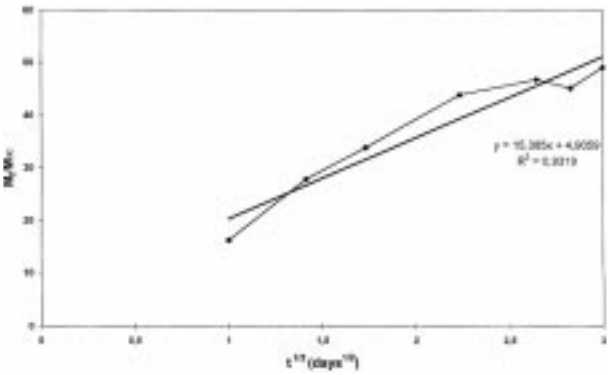

Figure 5 M_t/M_∞ for PLA 20 000 (10%) as a function of the square root of time.

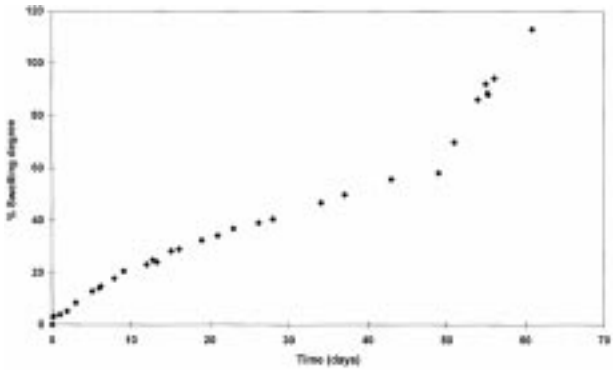

Figure 6 Degree of swelling for PLA 20 000 samples as a function of time.

(Fig. 7). It is evident that the higher pefloxacin level during the first six days, the most critical of the treatment, is located in the tibial cortex.

It is clear that drug delivery reaches a maximum on the 15th day (tibial cortex) and then gradually decreases, which is in agreement with the results from the *in vitro* study (Fig. 1). It is also evident that in the later stages of release, the drug concentration drops to the level of the first stage. The low pefloxacin concentration measured in the early stages of release shows that some diffusion of the drug occurs through the matrix of PLA. Degradation of PLA starts from the day six and consequently a sharp increase in the release rate is observed.

The release of pefloxacin lasted for 33 days, since PLA was totally degraded by that time. Also pefloxacin levels gradually decrease as we move away from the central point of the wound. It is also evident that both, *in vivo* and *in vitro* release (PLA 2000) give maximum pefloxacin concentration around the 15 day. The *in vivo* study lasted for 33 days whereas *in vitro* lasted for 56 days. This could be attributed to the fact that once the implant was placed in a bone cavity a local accumulation of macrophages, lymphocytes and giant cells occurred which resulted in an increase of the matrix degradation rate.

The pefloxacin released in the skin (Fig. 8) and the muscles (Fig. 9) reached its peak in the first 24 h, and its concentration in blood and in cancellous bone was zero. The serum concentration of pefloxacin remained at zero throughout the study, therefore no systemic side-effects were observed. Furthermore, no signs of inflammatory wound reaction appeared.

Regarding the clinical relevance of these results in

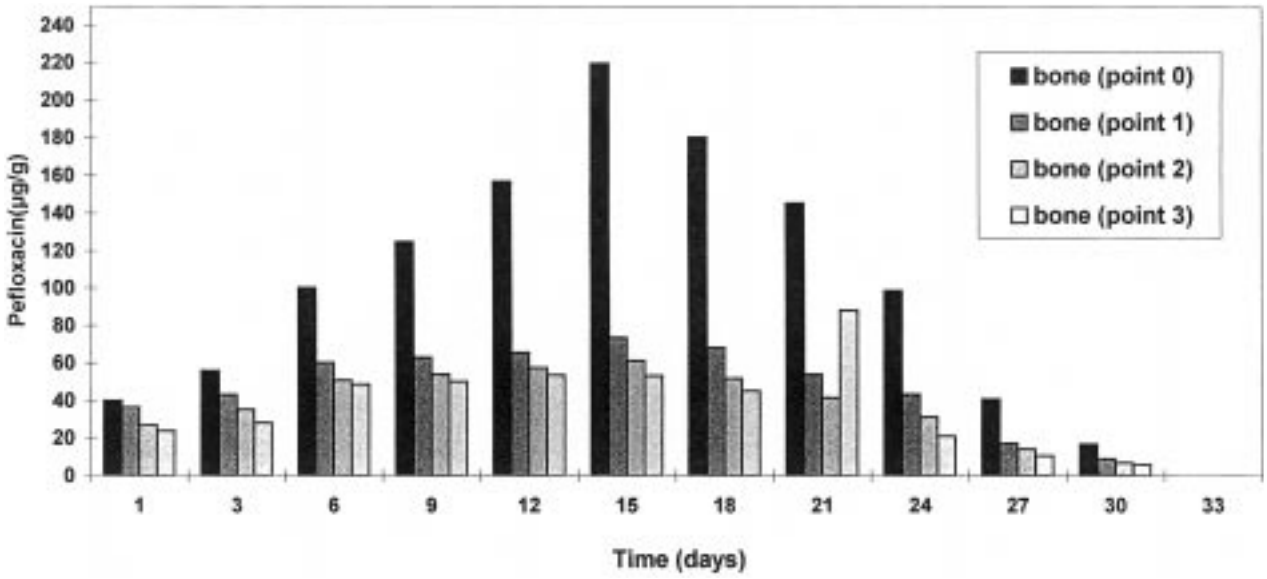

Figure 7 Pefloxacin concentration in the tibial cortex over 33 days post operation.

TABLE I Mean pefloxacin levels at various locations

Day of sacrifice	Skin (μg/g tissue)	Muscle (μg/g tissue)	Bone point 0 (μg/g bone)	Bone point 1 (μg/g bone)	Bone point 2 (μg/g bone)	Bone point 3 (μg/g bone)
1	10.56	29.42	39.91	36.42	26.92	23.89
3	8.04	25.32	55.86	43.23	35.19	28.09
6	5.83	23.40	100.2	60.36	50.86	48.71
9	4.06	21.26	124.82	63.13	53.96	50.2
12	2.98	19.01	156.75	65.61	57.53	53.54
15	2.36	16.18	219.68	73.54	61.36	53.16
18	1.76	11.81	180.51	68.16	51.86	45.15
21	0.84	8.18	145.35	53.91	41.50	88.35
24	0.50	5.91	98.63	43.34	31.30	21.15
27	0.13	2.83	40.83	17.15	14.18	10.7
30	0.60	1.2	16.80	9.15	6.92	5.87
33	0.02	0.06	0.07	0.01	0.01	0

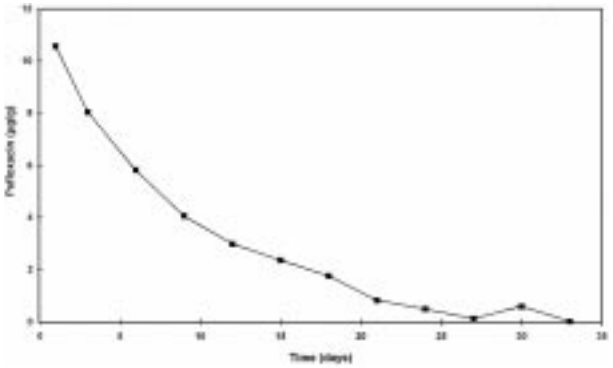

Figure 8 Pefloxacin concentration in skin over 33 days post operation.

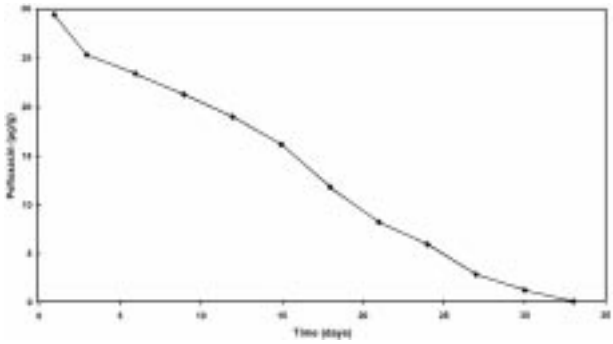

Figure 9 Pefloxacin concentration in muscles over 33 days post operation.

terms of antibiotic concentration, it should be noted that drug doses delivered exceed the Minimum Inhibitory Concentration (MIC) for most pathogens.

4. Conclusions

The results from the *in vitro* and *in vivo* studies suggest that PLA can be used as a drug carrier for the design of a controlled release system. The release in such systems can be described by a Fickian diffusion mechanism only for the first few days; then a degradation mechanism dominates in a second stage. The delivery of pefloxacin lasts four weeks and its concentration remains higher than the MIC. Systemic side-effects and local tissue reaction were satisfactory post-operatively.

The release properties of implants can be adjusted by using PLA with a molecular weight of 20 000. In this case polymer erosion can be ignored since it proceeds slowly for more than five months and the diffusion mechanism is predominant. In this way, drug delivery is prolonged and goes on for more than three months, which makes it interesting to investigate the kinetics of the *in vivo* release from a PLA20 000-pefloxacin.

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