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Evaluation of poly(lactic acid) as a biodegradable drug delivery system for parenteral administration

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Summary

Poly(lactic acid) (PLA) microspheres of 1–10 μm diameter prepared by emulsion deposition and containing entrapped prednisolone released the drug rapidly into an aqueous medium. Similarly sized microparticles prepared by a fusion process exhibited a more prolonged drug release profile and may have potential as a long-acting parenteral delivery system. Both methods of fabricating the polymer produced material which was cytotoxic when phagocytosed by mouse peritoneal macrophages. The intracellular toxicity and hence potential irritancy in vivo was only partially overcome by incorporating anti-inflammatory drug. Compressed implants of the same polymers containing prednisolone 10% w/w (100 mg · cm^{-3}) and weighing 12 mg were readily administered and sustained the delivery of the drug for over 30 days without complications at the implantation site.

Introduction

The role of biodegradable polymeric carriers in controlled drug delivery is the subject of several reviews (Wood, 1980; Deasy, 1984; Heller, 1984). Aliphatic polyesters and their copolymers have been tested extensively as implants, particles and microspheres for the delivery of narcotic antagonists, contraceptives, local anaesthetics, cytotoxics and anti-malarial agents.

Poly(lactic acid) (PLA) is reported to exhibit excellent biocompatibility at subcutaneous and other sites (Kulkarni et al., 1966; Cutright et al., 1971). However, Cutright and Hunsuck (1971) did note that PLA sutures produced a mild inflammatory response not caused by non-degradable material. Furthermore, Gurny et al. (1981) observed tissue necrosis in acute studies on intramuscular injections of PLA latex. In recent studies PLA microparticles exhibited poor biocompatibility in that they produced an inflammatory response when tested in vivo by the intra-articular route (Ratcliffe et al., 1984). The present work further examines the suitability of PLA as a biodegradable drug carrier, in the form of microparticles, microspheres and implants.

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Materials and Methods

Preparation of the polymer

Synthesis was based upon the method of Kulkarni et al. (1971). The cyclic dimer lactide

was prepared from 200 ml (±)-lactic acid (Fisons, Loughborough) by reflux at 140°C with 2% w/v zinc oxide at 60 mm Hg pressure reducing to 25 mm Hg over six hours. The product was distilled off at 200°C and 2 mm Hg then recrystallised at least twice from acetone. Polymerization was performed in an evacuated flask (0.01 mm Hg) at 150–180°C over 3 h in the presence of 0.2% tetraphenyl tin. PLA was dissolved in 1,4-dioxan and precipitated into water. The dried polymer was a creamy-white particulate solid with a melting range of 158–164°C, heat of fusion 3.7×10^4 $J \cdot kg^{-1}$ and an approximate molecular weight (Mw) of 100,000 as determined viscometrically in dioxan (Nuwayser et al., 1976). No residual lactide was apparent by thermal analysis (Perkin Elmer DSC 2B) or gel permeation chromatography (coupled Shodex eluted with tetrahydrofuran and detected by Waters R401 differential refractometer).

Preparation of the microspheres / microparticles

Microspheres in the size range 1–10 μm were prepared from a chloroform emulsion containing PLA in the dispersed phase and 1% w/v Tween 80 in the continuous phase. Glycerin (10% w/v) was added and the PLA deposited by nitrogen sparging.

Alternatively, microparticles of 1–10 μm were produced by thorough grinding of the glassy solid formed by the cooling of molten (170°C) PLA.

Drug-loading of the above preparations was achieved by introducing appropriate amounts of prednisolone into the dispersed chloroform phase or directly into the melt.

Preparation of implants

Implants were manufactured by direct compression of the above materials using a Manesty E2 single-punch tablet press fitted with 2.4 mm diameter tooling. Each implant weighed 12 ± 1 mg and measured 2.7 mm in length, hence being suitable for subcutaneous administration through an implantation instrument (Boots).

In vitro studies

The macrophage culture system employed was essentially the same as that described by Schorlemmer et al. (1977). Resident peritoneal macrophages from MF1 mice were plated out in petri dishes and cultured for 24 h prior to the addition of the polymer preparation at various concentrations. After a further 24 h cell morphology was examined using an inverted microscope. The culture medium was removed and the cells washed with buffered saline and disrupted with 0.1% Triton X-100. Samples of culture medium and cell extract were analyzed for the cytoplasmic enzyme lactate dehydrogenase (LDH) and the lysosomal enzyme β-glucuronidase (BG). Where zymosan was used to stimulate the macrophages to release lysosomal enzymes, the agent and polymer preparation were added simultaneously.

In vitro dissolution tests were performed on 100 mg of each preparation in 80 ml of pH 6.8 buffer (sink conditions) maintained at 37°C on a shaking water bath. Prednisolone was assayed at 242 nm spectrophotometrically or by high-pressure liquid chromatography.

In vivo studies

PLA implants (4 per animal, 2 either side of the mid-line) were injected subcutaneously into the flanks of Charles River CD rats, using an implantation instrument (Boots). The implants were removed at predetermined intervals, each site being examined for host response.

Recovered implants were inspected for gross deterioration, then dried and weighed. After rinsing each implant with distilled water the prednisolone content was determined spectrophotometrically in 1,4-dioxan.

Results and Discussion

Microparticles and microspheres: in vitro drug release

In vitro drug release profiles (Fig. 1) showed that microspheres released prednisolone at a relatively fast rate. In a previous study on PLA microspheres (0.45 μm) it was found that 50% of testosterone was released within 12 h in vitro (Gurny et al., 1981). Larger microspheres (50 μm) have been used to deliver progesterone (Beck et al., 1979) or local anaesthetics (Wakiyama et al., 1982) for up to 15 days but attempts at longer

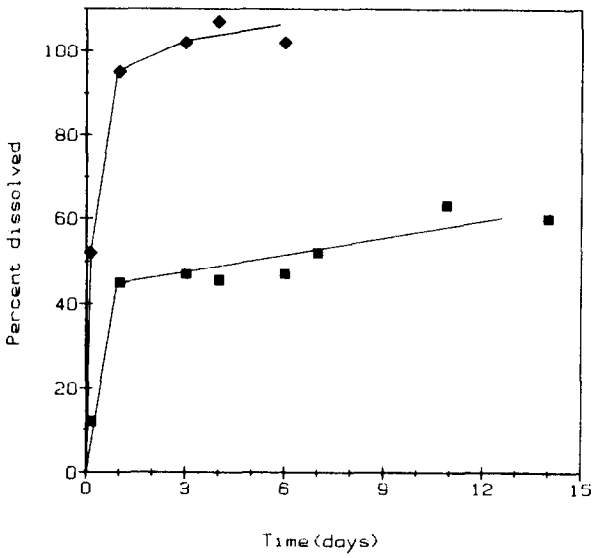

Fig. 1. Prednisolone release from microspheres and microparticles. ◆, microspheres; ■, microparticles.

periods of drug delivery have necessitated the use of PLA beads and/or coating with drug-free polymer (Mason et al., 1976; Nuwayser et al., 1976; Schwope et al., 1975; Pitt et al., 1976). In contrast to the microspheres, the in vitro release profile for prednisolone from microparticles was remarkably prolonged (Fig. 1). After an initial rapid escape of drug presumably from at or near the particle surfaces, the prednisolone continued to be released at a slow rate for over 60 days. This indicates that the fusion process may have substantial advantages for thermo-stable drugs requiring long-term release.

Scanning electron microscopy suggests a possible explanation for the different in vitro release characteristics of microspheres and microparticles. The microspheres appeared smooth but possessed a highly porous internal structure characteristic of PLA-prednisolone produced by the evaporation of solvent. Crystals of drug could be readily identified within the polymer matrix and it is likely that the open structure of PLA allows relatively rapid release of prednisolone by diffusion from the microspheres. Microparticles from the melt process exhibited a dense amorphous structure which presents a greater barrier to the ingress of solvent and impedes the release of the drug by diffusion but it would also appear likely that polymer degradation plays some part in controlling the release of prednisolone from the microparticles.

Microparticles and microspheres: in vitro cell culture

Although microparticles from the fusion process appear to offer possibilities as injectable sustained-release vehicles, PLA has previously been found to be irritant when administered to rabbits by the intra-articular route (Ratcliffe et al., 1984).

One means of testing the potential irritancy of microparticulate substances is to examine their stimulatory effect on macrophages in cell culture (Schorlemmer et al., 1977). These workers found good correlation between stimulation of macrophages as judged by lysosomal enzyme release, and irritancy in vivo. Since non-degradable microspheres (polystyrene latex) were found to be well tolerated (Schorlemmer et al., 1977) we have tested PLA microspheres in a similar manner.

Microspheres of PLA were readily phagocytosed by macrophages both in the presence and absence of the phagocytic stimulus zymosan (25 $\mu g \cdot cm^{-3}$). Analytical results on the cell extract and culture medium are summarized in Table 1. Although the reduction in BG synthesis, indicated by a fall in total enzyme, is potentially of therapeutic significance, the release of quantities of this lysosomal enzyme in the absence of zymosan suggests that the polymer may be cytotoxic. This was confirmed by the release of cytoplasmic enzyme (LDH) at all polymer concentrations. At 50 μg/ml dead cells were visible in the culture and at higher polymer levels all macrophages were killed. Effects were similar in the presence of zymosan although cytotoxicity was more marked, presumably due to a cumulative effect since zymosan itself is toxic at high concentrations. These observations suggest that polymer particles might be irritant in vivo.

PLA preparations containing prednisolone were examined to determine whether the presence of an associated anti-inflammatory agent could outweigh the potential irritancy of the polymer. From Fig. 1 it would appear that microparticles have the greater potential for drug delivery and these were, therefore, examined in cell culture.

TABLE 1

EFFECT OF PLA MICROSPHERES ON MOUSE PERITONEAL MACROPHAGES

Culture	Polymer conc. ($\mu g \cdot cm^{-3}$)	Induced change in enzyme (%)					
		LDH			BG		
		Cell	Medium	Total	Cell	Medium	Total
U	12.5	−4	+213	+13	−70	+133	−51
U	25	−30	+715	+30	−78	+244	−48
U	50	−61	+790	+7	−82	+622	−16
U	75	–	–	–	–	–	–
S	12.5	−49	+331	−14	−71	+31	−35
S	25	−62	+456	−15	−77	+20	−43
S	50	–	–	–	–	–	–
S	75	–	–	–	–	–	–

U = unstimulated cells; S = cells stimulated with 25 $\mu g \cdot cm^{-3}$ zymosan; – = cells dead, plates discarded; LDH = lactate dehydrogenase; BG = β-glucuronidase.

Sufficient drug was present to produce up to 10^{-4} $mol \cdot dm^{-3}$ in the culture medium and since prednisolone inhibited zymosan induced lysosomal enzyme release at 10^{-6} $mol \cdot dm^{-3}$, some demonstrable effect on the culture would be predicted. Little cytotoxicity was associated with drug-loaded polymer at 6.25 $\mu g \cdot cm^{-3}$ (Table 2). The remaining polymer concentrations still produced a fall in BG synthesis. Some lowering of LDH release relative to drug free microspheres was observed. Despite these effects on the macrophages the potential irritancy of the polymer was not overcome although it is clearly possible that additional anti-inflammatory agent might suppress the response.

Implants

In view of the previous use of PLA for drug delivery and the potential irritancy of the polymer indicated above, it would seem essential to examine the use of PLA in the form of implants.

The drug release profiles for compressed implants are shown in Fig. 2. Each implant contained 10% w/w prednisolone representing 100 $mg \cdot cm^{-3}$. The amounts of drug released in vitro were approximately linear with the square-root of

TABLE 2

EFFECT OF PLA MICROPARTICLES CONTAINING PREDNISOLONE ON MOUSE PERITONEAL MACROPHAGES

Culture	Polymer conc. ($\mu g \cdot cm^{-3}$)	Induced change in enzyme (%)					
		LDH			BG		
		Cell	Medium	Total	Cell	Medium	Total
U	6.25	−10	+93	−0.4	−3	+26	+0.3
U	12.5	−25	+290	+4	−44	+201	−18
U	25	−28	+323	+4	−48	+236	−18
U	50	−33	+186	−13	−48	+108	−32
S	6.25	−10	+28	−4	+10	−13	−2
S	12.5	−55	+193	−20	−42	+0.5	−21
S	25	−53	+233	−12	−45	+14	−16
S	50	−63	+168	−30	−47	−13	−31

U = unstimulated cells; S = cells stimulated with 25 $\mu g \cdot cm^{-3}$ zymosan; LDH = lactate dehydrogenase; BG = β-glucuronidase.

time and for convenience all the data on implants are expressed in this way. Compressed microspheres released about 9% of entrapped prednisolone over 4 days and only 20% after 2 weeks. Nevertheless, this was a significantly faster rate ($Q/t^{0.5} = 0.4$ mg/cm^2/day$^{0.5}$) than the implants from microparticles ($Q/t^{0.5} = 0.2$ mg/cm^2/day$^{0.5}$). The plot for the latter preparation conceals excellent zero-order characteristics followed by a progressive increase in the release rate after about 70 (and beyond 100) days in vitro, suggesting that drug delivery is at least partially dependent upon polymer degradation.

Whilst results obtained on recovered implants are necessarily less precise certain trends in behaviour are clear. The root-time relationship was less linear in vivo, furthermore the two types of implant showed lesser differences (Fig. 2) and release occurred more rapidly ($Q/t^{0.5}$ between 0.5 and 0.6 mg/cm^2/day$^{0.5}$). This may indicate faster penetration by biological fluids and/or more appreciable degradation of PLA in vivo. Nevertheless, drug release from both polymer presentations was easily sustained over the period of the study

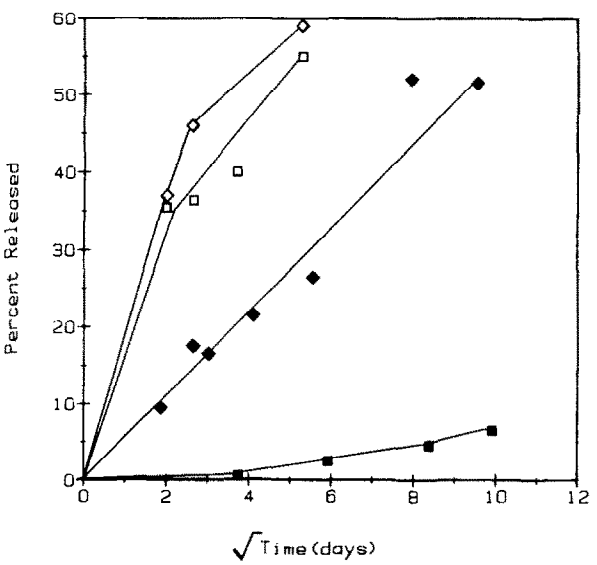

Fig. 2. Prednisolone release from implants. ◇, compressed microspheres in vivo; □, compressed microparticles in vivo; ◆, compressed microspheres in vitro; ■, compressed microparticles in vitro.

and an initial "burst" of released drug (35% in 4 days) might be clinically advantageous in some applications. The implanted systems were readily recoverable after 28 days with up to 15% loss of dry weight and no visible gross deterioration, encapsulation or inflammation.

General discussion

Prolonged drug release profiles have been obtained from PLA microparticles prepared by a fusion technique. Release from the more porous microspheres produced by a process involving solvent evaporation was too rapid for practical use. However, sustained drug release was obtained from compressed implants prepared from either type of PLA.

Microspheres and microparticles were shown to elicit a cytotoxic effect on macrophages in culture. Similar cytotoxic effects are known to occur with high concentrations of zymosan which has also been shown to produce an intense inflammatory response in vivo when injected intra-articularly (Keystone et al., 1977), or into the ham-string muscle (Schorlemmer et al., 1977) of mice. Cytotoxicity and the release of lysosomal enzyme and LDH from macrophages are also implicated in the inflammatory response to monosodium urate crystals (McMillan et al., 1981). The observed behaviour of drug-free or drug-loaded PLA particles in vitro is therefore entirely consistent with, and probably explains the inflammatory effects reported in vivo (Ratcliffe et al., 1984). The mechanism of the response to PLA may be related to the acute synovitis induced by crystalline steroids (McCarty and Hogan, 1964) and the inflammatory action of hydroxyapatite crystals (Schumacher et al., 1977). The cytotoxic effects of PLA are unlikely to be due to the morphology of the polymer or particle shape since both microspheres and microparticles elicited similar response (Tables 1 and 2). Intracellular toxicity may depend upon a large number of factors such as particle size, electrostatic charge, molecular weight, degradation rate, degradation products or impurities such as the traces of catalyst which are commonly present in PLA. Significantly, neither PLA preparation

appeared to be bio-incompatible in the form of compressed implants at subcutaneous sites in mice. This indicates that the irritancy of PLA microspheres and microparticles is a function of their internalisation by macrophages. In view of the excellent drug release characteristics of the microparticles, further work is justified to elucidate the mechanism of toxicity and reduce its effect.

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