The response of macrophages to particles of resorbable polymers and their degradation products

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Alpha polyesters such as poly(L-lactide) and poly(glycolide) are biodegradable materials used in fracture fixation and they need to be assessed for problems associated with their degradation products. This study has compared cell responses to low molecular weight poly(L-lactide) particles, lactate monomer, poly(glycolide) particles and glycolic acid at cytotoxic and sub-cytotoxic concentrations. Murine macrophages were cultured *in vitro* and the release of lactate dehydrogenase (LDH), prostaglandin E_2 (PGE_2) and interleukin-1 alpha IL-1α was measured following the addition of particles or monomer. Experiments revealed that both the poly(L-lactide) and poly(glycolide) particles gave rise to dose dependent increases in LDH release and an increase in IL-1α and PGE_2 release. Comparisons of the poly(L-lactide) particles to the poly(glycolide) particles did not reveal any differences in their stimulation of LDH, IL-1α and PGE_2 release. The lactate and glycolate monomers did not increase PGE_2 or IL-1α release above control levels. There was no difference in biocompatibility between the poly(L-lactide) and poly(glycolide) degradation products both in particulate and monomeric form.

1. Introduction

Poly(glycolide) and poly(L-lactide) are amongst the most common materials used to manufacture bioresorbable fracture fixation devices [1]. Although few complications have been reported to date with the use of these materials, the most frequent problem encountered (an incidence of approximately 5%) [2] is the development of a late foreign body inflammatory reaction. This may be the cause of the osteolysis seen around some rods and screws in a small number of patients [3]. Often the bursitis associated with these implant materials is painful and requires drainage. Such problems have been more commonly associated with the fast degrading poly(glycolide) however poly(L-lactide) implants, which degrade over a period of around six years, can give rise to similar symptoms with time [4]. Studies of biopsy specimens and the degradation of these polymers suggest that the onset of problems is directly related to implant degradation including the generation of small particles [5]. The presence of wear particles from other orthopaedic devices has been shown to trigger a foreign body reaction whereby cells such as macrophages produce cytokines and prostaglandins that induce inflammation and bone resorption [6–8]. It is not clear how debris generated during resorbable implant degradation affects the surrounding bone and tissue in terms of its inflammatory and osteolytic potential.

The smallest resorbable particles will eventually degrade into the monomeric units; lactate in the case of poly(L-lactide) and glycolate in the case of poly(glycolide). A decrease in pH and increase in osmolarity at the implant site is associated with the release of these acidic compounds and could be deleterious [1, 9, 10]. There is evidence that high molecular weight poly(L-lactide) material (as is used for orthopaedic implants) can undergo heterogeneous bulk degradation and may collapse releasing degradation products that were previously trapped within a fragile external ''shell'' [11, 12]. Poly(L-lactide) and poly(glycolide) need to be assessed for problems associated with their degradation products. This requires studies on the effects of intact and fragmented implants, particles and monomers.

This study has compared the biocompatibility of low molecular weight poly(L-lactide) particles and lactate

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TABLE I Osmolarity measurements of monomer and control solutions. Each measurement is the mean ± sd of the osmolarities measured on three separate occasions. (Media control = 286 ± 2)

Monomer	Concentration (mM)			
	5.55	11.1	27.8	55.5
Lactate	292 ± 3	303 ± 7	325 ± 4	369 ± 2
Glycolate	299 ± 5	300 ± 6	326 ± 16	375 ± 14
NaCl	—	—	318 ± 18	358 ± 26
Sucrose	—	—	315 ± 1	356 ± 25

monomer to poly(glycolide) particles and its monomer at cytotoxic and sub-cytotoxic concentrations by measuring cell viability and the release of the bone resorbing and inflammatory mediators, prostaglandin E_2 (PGE_2) and interleukin-1 alpha IL-1α from murine macrophages *in vitro*.

2. Materials and methods

2.1. Cell culture

Murine macrophages of the cell line IC21 (ECACC, Porton Down, UK) were cultured using RPMI 1640 cell culture medium containing 10% (v/v) foetal calf serum and 1% (v/v) glutamine. Cells were allowed to reach 80% confluency before they were washed with phosphate buffered saline, split using a non-enzymatic cell dissociation solution and seeded into test wells.

2.2. Preparation of lactate and glycolate solutions

RPMI cell culture medium supplemented with 10% (v/v) foetal calf serum and 1% (v/v) glutamine was used in the negative controls. Media containing lactate and glycolate were made by serial dilution of cell culture medium containing 111 mM lactate or glycolate to give solutions with a final concentration of 55.5, 27.8, 11.12, 5.56 and 2.78 mM. The pH of 111 mM glycolate and lactate stock solutions was adjusted to pH 7.4 using 1 M NaOH to eliminate the effect of variations in pH. Media containing 55.5, 27.8 and 11.12 mM concentrations of NaCl or sucrose, which exhibited similar osmolarity to identical concentrations of lactate or glycolate, were used to control for the effects of changes in osmolarity (Table I). All media were filter sterilized prior to use.

2.3. Preparation of particle solutions

Low molecular weight poly(L-lactide) and poly(glycolide) particles were produced by grinding materials RL104 (Mw 2000) and RG110 (Mw 6000) respectively. These materials were obtained from Boehringer Ingelheim, Ingelheim, Germany and have been characterized previously [13]. The poly(glycolide) particles had a median diameter of 21.77 μm (10% were less than 4.39 μm) and the poly(L-lactide) particles had a median diameter of 15.09 μm (10% were less than 2.02 μm). Particles were sterilized by exposure to ethylene oxide then resuspended in cell culture medium at the following concentrations: 0.01, 0.05, 0.1, 0.5 and 1 mg/ml and briefly vortexed before addition to cells. These concentrations cover a non-toxic to a cytotoxic range as previously determined [9].

2.4. Endotoxin measurement

Particles of poly(L-lactide) and poly(glycolide) were suspended in endotoxin free water at a concentration of 1 mg ml^{-1}, brought to neutral pH with 0.05 M TrisHCl buffer (pH 7.4) and tested for the presence of bacterial endotoxin using the Kinetic-QCL™ limulus amoebocyte lysate assay with a sensitivity of 0.005 endotoxin units per ml (Biowhittaker, Wokingham, UK).

2.5. *In vitro* test method

IC21 cells were seeded into 24-well plates at a density of 4×10^4 cells per cm^2. Test solutions were added to the plates 24 h later, following removal of the culture medium. Supernatants and cell lysates were harvested from all wells at 24, 48 and 72 h, centrifuged to remove cell debris and stored at −70 °C prior to analysis.

Cytotoxicity was measured using a lactate dehydrogenase assay (Cytotox 96, Promega, Southampton, UK), as described previously [13]. A competitive enzyme immunoassay system measured PGE_2 release (BIOTRAK Prostaglandin E_2 EIA, Amersham Life Science, Amersham, UK) and a non-competitive sandwich enzyme-linked immunosorbent assay was used to determine levels of IL-1α (Endogen, Woburn, MA).

2.6. Statistics

Linear regression was used to assess the effect of concentration, time and particle or monomer type upon IL-1α, LDH and PGE_2 release. In the case of monomers the dose-response was modeled linearly whilst for particles, release was better modeled as increasing linearly with the logarithm of concentration. Statistical analysis was performed on the pooled data from all time points. In all cases, time was included in the model before assessing the effect of substance type. The effect of time was assessed after controlling for concentration, type and the concentration-by-type interaction, when these were significant predictors of release. Analysis of the residuals showed no substantial deviation from normality. Multiple significance tests were carried out which increases the possibility of achieving significance by chance. Thus only values of $p = 0.01$ or less were considered significant.

3. Results

3.1. Time course

Cytokine and LDH release were measured at 24, 48 and 72 h. The effect of time on the absolute amount of release and on the strength of the dose response was investigated. There was a significant increase in the amount of IL-1α released with time, stimulated by both particles ($p = 0.01$) and monomer ($p < 0.0001$). There was also an increase in the strength of the dose response to particles ($p < 0.0001$) and monomer ($p < 0.0001$) in the release of IL-1α. There was no significant effect of time on PGE_2 or LDH release. Although time affected the release of IL-1α, the direction of the response was the same at each time point and the statistical tests were performed on all data pooled. The 24 h data have been presented graphically to illustrate the findings (Figs. 1 and 2).

3.2. Particle Experiments

Bacterial endotoxin was not detected in either the poly(L-lactide) or poly(glycolide) particle suspensions. There was a dose dependent effect of particle concentration on the release of IL-1α ($p < 0.0001$), PGE_2 ($p < 0.0001$) and LDH ($p < 0.0001$) with both particle types (Fig. 1). There was no difference in the response to poly(L-lactide) and poly(glycolide) particles ($p = 0.117$, p = 0.767 and $p = 0.986$ for IL-1α. PGE_2 and LDH release respectively).

3.3. Monomer Experiments

The was no dose dependent effect of monomer addition on IL-1α or PGE_2 release ($p = 0.498$ and $p = 0.909$ respectively) but LDH release was affected by the concentration of glycolate ($p = 0.0002$) (Fig. 2). There was no difference between the release of IL-1α and PGE_2 stimulated by lactate or glycolate monomer ($p = 0.081$ and $p = 0.879$ respectively). Although glycolate increased LDH release and lactate did not, at the level of significance accepted in this study there was no significant difference between the effect of glycolate and lactate on LDH release ($p = 0.043$).

4. Discussion

The particle experiments revealed that both the poly(L-lactide) and poly(glycolide) particles gave rise to dose dependent increases in LDH release. The LDH results

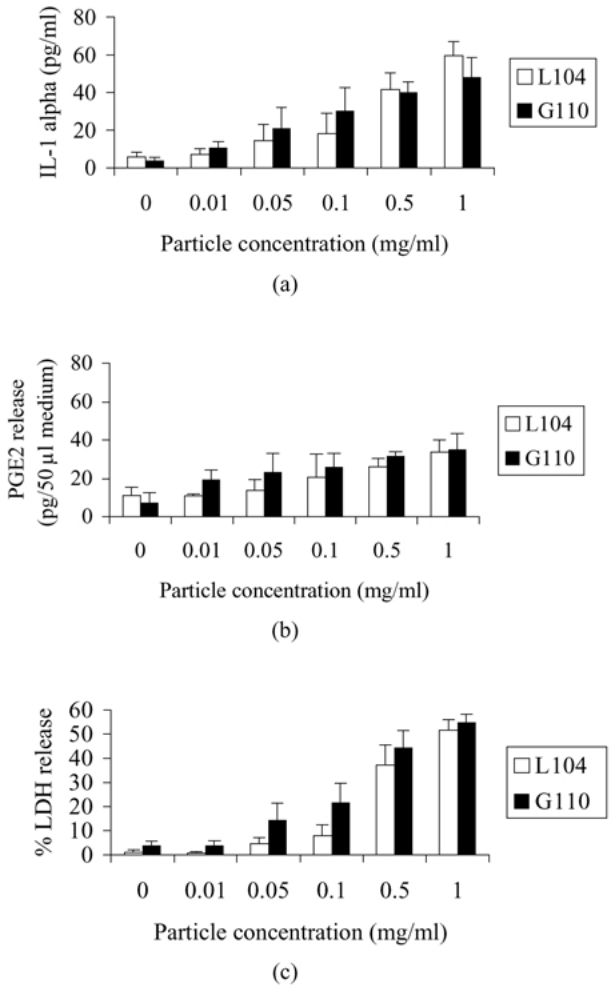

Figure 1 IL1-α (a), PGE_2 (b) and LDH (c) release from IC21 macrophages following exposure to poly(L-lactate) (L104) and poly(glycolate) (G110) particles for 24 h.

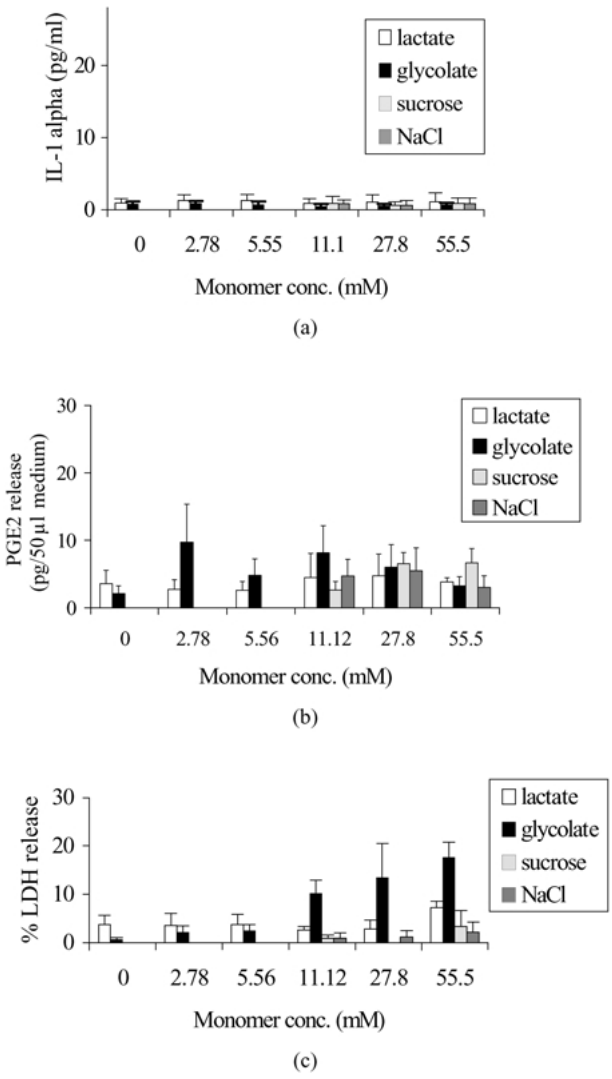

Figure 2 IL1-α (a), PGE_2 (b) and LDH (c) release from IC21 macrophages following exposure to lactate and glycolate monomer for 24 h.

compare favorably with our previous work [13] where it was demonstrated that concentrations of 0.5 mg/ml RL104 and G110 produce a significantly increased release of LDH from macrophages. The major finding from these experiments is that both low molecular weight poly(L-lactide) and poly(glycolide) particles can increase IL-1α and PGE_2 release from macrophages *in vitro* and to our knowledge this is the first time that has been reported. It is well known that IL-1α and IL-1β are important molecules involved in bone resorption [14] and inflammation [15] and it has been demonstrated that infusion of IL-1 into the joint space can produce the type of destructive changes seen in arthritis [16]. Many of the effects of IL-1 are due to the induction of arachidonate metabolism with molecules such as PGE_2 and leukotriene B4 functioning as second messengers [17–19]. Other particulate biomaterials increase IL-1 and PGE_2 release, for example ultra high molecular weight polyethylene, titanium alloy, and cobalt chrome alloy [20] but some particulates have been shown to reduce IL-1α release, for example hydroxyapatite and fluorapatite [21]. Direct comparisons of the poly(L-lactide) particles to the poly(glycolide) particles did not reveal any differences between them in terms of LDH, IL-1α and PGE_2 release.

Increasing concentrations of glycolate monomer produced increases in LDH release but increasing concentrations of lactate monomer had no effect. Although the NaCl and sucrose solutions had a similar osmolarity to the glycolate solutions at 55.5 mM concentrations, they did not elicit the same increase in LDH release. Therefore the LDH increases observed were due to effects of the glycolate monomer other than associated osmolarity changes.

The lactate and glycolate monomers did not increase PGE_2 or IL-1α release above controls in these experiments. Although the greatest concentration of monomer used in this study was 55.5 mM, in a previous study [9] we found that 10 mg/ml (111 mM) of lactate monomer significantly increased PGE_2 production by macrophages above control levels. This was thought to be mainly due to cell lysis. Our previous work suggested that fibroblasts were more likely to release PGE_2 in response to lactate monomer than macrophages and in future it may well be worth investigating whether the same is true for glycolate monomer.

In conclusion, there was a dose dependent increase in IL-1α and PGE_2 release caused by particulate poly(L-lactide) and poly(glycolide). This lends support to the theory that the particles produced as poly(glycolide) implants degrade can cause an inflammatory response [5]. As there was no difference between the response found with poly(L-lactide) and poly(glycolide) particles, this would suggest that the swellings associated with poly(glycolide) implants could also be found with more the slowly degrading poly(L-lactide). Although long term follow-up of poly(L-lactide) implants is limited, there is some evidence that this is the case. Tissue responses to poly(glycolide) generally manifest themselves at about 11 weeks after surgery whereas foreign body reactions to poly(L-lactide) have been seen 4–5 years after fracture fixation [2]. A recent case study of a 63 year old woman described a delayed aseptic swelling 15 months after fixation of a talar neck fracture with a biodegradable poly(L-lactide) rod [22]. Other experiments involving mandibular osteotomies in sheep have revealed that although foreign body reactions were generally mild, the particles of poly(L-lactide) polymer could still be detected at the implantation site at 5 years [23]. Experiments where poly-L/-D lactide implants were inserted subcutaneously into rabbits demonstrated that tenascin and cellular fibronectins were present at the implant interfaces and that myofibroblasts were also upregulated when implant hydrolyzation began; this suggested that the poly-L/-D lactide implants produced a prolonged period of wound healing [24]. Other authors have suggested that large implants made of poly(L-lactide) should not be used for insertion through intra-articular surfaces since the long degradation time can impede the restoration of articular cartilage in the rabbit femur [25]. These studies suggest that more long term research into the effects of poly(L-lactide) resorption *in vivo* is required. It will certainly be interesting to investigate whether the levels of IL-1α released from macrophages in response to these resorbable particles are sufficient to up-regulate PGE_2 release by periprosthetic fibroblasts.

Acknowledgments

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