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Preparation and evaluation of biodegradable polymeric systems for the intra-articular delivery of drugs

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Colloidal suspensions of four biodegradable polymers, polylactic acid (PLA), polybutylcyanoacrylate (PBCA), gelatin (PG) and albumin (PA) were prepared within the size range 1–10 μm. In-vivo biocompatibility tests with synovial tissues were carried out to assess the irritancy of the polymers following intra-articular injection into rabbit knee joints. PLA, PBCA and PG were found to cause joint inflammation whereas PA was well tolerated by the tissues. PA microspheres may provide a means of sustaining the release and reducing the rate of clearance of drugs from the knee joint.

The intra-articular injection of drugs for the treatment of rheumatoid arthritis is complicated by the rapid clearance of the drug from the joint into the circulation, with resulting systemic effects. For example, following intra-articular administration of corticosteroids, suppression of plasma cortisol (Bird et al 1979) and a decrease in the number of circulating eosinophils (Mason & Ward 1953) have been observed.

Many attempts have been made to improve the retention of steroids within the joint cavity, including the use of less soluble esters, such as the tertiary butyl acetate (Hollander 1955) and microcrystalline preparations such as triamcinolone hexacetonide (Bird et al 1979). Colloidal systems have been employed to improve the retention of gold ^{198}Au and yttrium ^{90}Y following intra-articular injection for radiosynovectomy. Liposomes have also been used to improve the retention of methotrexate (Foong & Green 1983) and cortisol palmitate (Dingle et al 1978). In the latter case, although the amount of steroid injected could be reduced by the use of liposomes, the anti-inflammatory effect could be sustained only for 3–4 days (Shaw et al 1979) possibly due to rapid release of the steroid from the liposome.

We have investigated the feasibility of using biodegradable polymers as a means of achieving maximal retention and duration of action of drugs administered by the intra-articular route. Referring to the literature, there are a number of possible polymeric carriers that could be used. Polyalkylcyanoacrylate nanoparticles (Couvreur et al 1979) and microspheres of albumin (Morimoto et al 1981) or gelatin (Sezaki et al 1982) have been used previously, as a means of altering the systemic distribution of cytotoxic drugs within the body. Polylactic acid microspheres have been used to sustain the release of local anaesthetics (Wakiyama et al 1981).

In the present study, colloidal suspensions of polybutylcyanoacrylate, polymerized gelatin, polymerized albumin and polylactic acid have been prepared and their biocompatibility with synovial tissues assessed by determination of irritancy following intra-articular injection into rabbit knee joints.

METHODS

Preparation of polymers

Polylactic acid (PLA). PLA was prepared by modification of the method of Kulkarni et al (1971). Water was removed from 200 ml (±)-lactic acid (Fisons Ltd, Loughborough) by distillation with 2% w/v zinc oxide at 140 °C and reduced pressure (25 mm Hg) over 5 h. The resulting dimer, lactide, was distilled off at 200 °C (2 mm Hg), recrystallized from ethylacetate and washed with carbon tetrachloride. 20 g lactide and 0·02% tetraphenyl tin were then heated in an evacuated, sealed flask at 150–180 °C (0·01 mm Hg) for 2 h. The resulting PLA was purified in dioxan with reprecipitation in water, then dried. The polymer was ground with a pestle and mortar until a particle size of 1–10 μm was obtained. PLA so produced was a free-flowing particulate solid with a melting point range of 160–170 °C, deter-

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mined by differential scanning calorimetry and a molecular weight of approximately 100 000–120 000, determined in dioxan, by viscometry (Nuwayser et al 1976).

Polybutylcyanoacrylate (PBCA). 3 ml methanol was added dropwise to 3 ml butylcyanoacrylate monomer (Loctite, Dublin) until precipitation of the polymer was complete. The PBCA was purified by recrystallization with hot acetone. Finally, the polymer was dissolved in acetone, partially dried in a rotaevaporator (30 °C) and the drying completed in a vacuum oven. The pure polymer was then ground with a pestle and mortar. Larger particles were removed from the sample by wet sieving through 30 and 10 μm mesh sieves to leave a product with a particle size range of 1–10 μm.

Gelatin (PG). Gelatin microspheres were prepared by modification of the method of Yoshioka et al (1981). 0·9 ml aqueous gelatin solution (30% w/v) (ossein gelatin, Croda Gelatins Ltd, Cheshire) and 9 ml corn oil were heated to 70 °C in a water bath. A water-in-oil (w/o) emulsion was then formed by 3 methods: (i) mixing (Whirlimixer, Fisons, type WM/250/SC), (ii) homogenization (Silverson homogenizer, type 17231), (iii) sonication (Dawe, type 7532B). On formation of the emulsion the gelatin spheres were gelled by cooling in ice and the oil phase removed by dilution with cold acetone. The spheres were then collected and dried by filtration, under vacuum, through a 1 μm polycarbonate membrane (Nuclepore) using a Sartorius membrane filter unit. The spheres were washed with further acetone and then cross-linked by continuous stirring for 10 min in a 30% v/v formaldehyde in acetone solution. The spheres were wet sieved through 30 and 10 μm mesh sieves and collected and dried by filtration, the product being a free-flowing powder.

The effect of a surfactant on the size distribution of the spheres was investigated by incorporation of 1–3% v/v Tween 20 (Honneywill-Atlas) into the oil phase before emulsification.

Albumin (PA). PA microspheres were produced by a modification of the method of Tomlinson et al (1982). 12·5% w/v rabbit serum albumin (Fraction V, Sigma, UK) in pH 7·6 phosphate buffer (0·2 ml) was added to 25 ml olivé oil. This was stirred for 5 min, under turbulent flow conditions, using a mechanical stirrer (Heidolph Type RR1) to form a w/o emulsion. 0·1 ml glutaraldehyde (BDH) solution (25% v/v) was added and the emulsion stirred for a further 15 min to denature and cross-link the albumin. The microspheres were collected by centrifugation at 2500*g* for 20 min. The oil was then removed and the spheres washed with light-petroleum (bp 40–80 °C) followed by ethanol. The microspheres were sieved through a 30 μm sieve and collected and dried by filtration using a 1 μm polycarbonate membrane. The effect of surfactant concentration (1–5% v/v Tween 20) and stirring speed on the size distribution of the PA microspheres was investigated.

Size analysis

All particles were sized using a Coulter counter (Model TA, Coulter Electronics, UK). The particles were resuspended in water together with 1% v/v Tween 20 and sonicated before counting.

Evaluation of biocompatibility

Six New Zealand White rabbits (2·5–3·0 kg) were used to test each polymer. 2·5 mg polymer (10 mg ml^{-1} in 0·05% v/v Tween 20 in 0·9% w/v NaCl (saline)) was injected into the right knee joint cavity, 0·25 ml of 0·05% v/v Tween 20 in saline being injected into the left joint cavity as a control. Within each group, 3 rabbits were killed at 3 and 7 days following injection of the polymer. The infrapatellar folds containing the synovial membrane and submembranous adipose tissue were removed, fixed in buffered formalin and embedded in paraffin wax. Sections (5 μm) were cut at four different levels through the block, stained with eosin and haematoxylin and evaluated for the following changes: (i) hyperplasia of the synovial lining cells, (ii) cellular infiltration of the subintima, (iii) deposition of collagen and fibrin in the subintima. Each of these parameters was scored on a 0–5 scale, a score of 0 representing normal tissue while that of 5 referring to a severe, generalized inflammatory response.

Table 1. Geometric size distributions of the polymers.

Polymer	Mean (μm)	s.d.
PBCA	3·5	1·74
PLA	7·4	1·84
PG	5·5	2·10
PA 1000 rev min^{-1}		
5·0% v/v Tween 20	2·2	1·60
2·0% v/v Tween 20	2·8	0·60
1·2% v/v Tween 20	3·1	0·50
0·4% v/v Tween 20	3·3	1·70
PA 2000 rev min^{-1}		
1·2% v/v Tween 20	1·9	1·50

RESULTS

Preparation of polymers

The particle sizes of the polymers showed log normal distributions and the results are summarised in Table 1. Grinding of PLA and PBCA polymers to a narrow size range less than 10 μm was difficult. Emulsification of the aqueous gelatin solution by mixing or homogenization produced gelatin microspheres of a wide size range. A particle size of less than 6 μm was achieved by sonication. This method was consequently chosen for the production of gelatin microspheres to be tested for tissue biocompatibility in-vivo. However, manufacture of microspheres by this method was difficult. The heat generated during the process was not easy to dissipate, and caused denaturation and aggregation of the microspheres at sonication times greater than 1 min. Incorporation of Tween 20 into the emulsion had little effect on the size distribution whatever method of emulsification was used.

Formation of albumin microspheres from a w/o emulsion was achieved only with rapid stirring of the reaction mixture. Incorporation of Tween 20 into the emulsion decreased the mean particle size. Combination of a low concentration of surfactant and of a rapid stirring speed resulted in a greater reduction of the mean particle size. A concentration of 1·2% v/v Tween 20 and a stirring speed of 2000 rev min^{-1} were therefore chosen for the production of albumin microspheres.

Biocompatibility of polymers

The histological results for the four polymers tested are summarized in Table 2. All control joint tissues were normal in appearance at 3 and 7 days post-injection (Fig. 1). Intra-articular administration of PLA particulates caused marked inflammation of the synovial membrane and underlying tissues. Three days post-injection a general hyperplasia of the synovial membrane was observed in all 3 rabbits (Fig. 2). After 7 days the hyperplasia became more marked and generalized with an increase in vascularity, fibroblast infiltration and collagen deposition within the subintima.

PBCA particulates caused a severe, localized inflammatory response in one rabbit from each group at 3 and 7 days post-injection (Fig. 3). This was characterized by hyperplasia of the membrane with infiltration by macrophages and polymorphs. The remainder of the membrane and tissues were virtually normal in appearance. The joint tissues of all other rabbits within the groups were found to be normal.

Injection of gelatin microspheres into the joint cavity caused a mild generalized hyperplasia of the synovial membrane and widespread diffuse cellular infiltration throughout the subintima. Deposition of collagen and fibrin was slight. One animal at 7 days

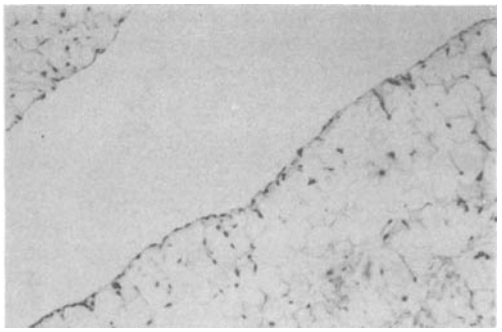

FIG. 1. Section through normal rabbit synovium 7 days after injection of 0·25 ml 0·05% v/v Tween 20 in normal saline (×160). Stain: Haematoxylin & eosin.

Table 2. Histological changes occurring in the synovium 3 and 7 days after intra-articular (i.a.) injection of polymers.

Polymer	Days after i.a. injection	Histological score* Hyperplasia	Cellular infiltration	Fibrosis	Total
PLA	3	1·5	0·0	0·0	1·5
	7	3·0	3·0	2·5	8·5
PBCA	3	2·0	2·0	2·0	6·0
	7	1·5	1·5	1·5	4·5
PG	3	2·5	3·0	2·0	7·5
	7	2·5	2·5	2·0	7·0
PA	3	1·5	0·0	0·0	1·5
	7	1·0	0·5	0·5	2·0

* Graded on 0–5 scale.

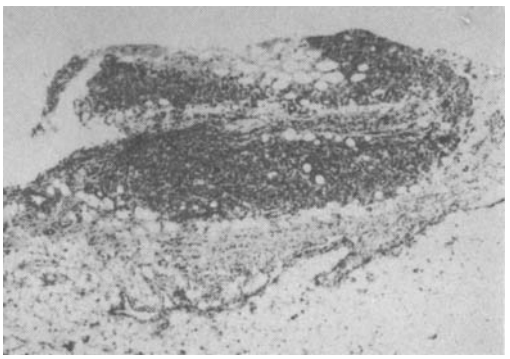

FIG. 2. Section through rabbit synovium 7 days after injection of 2·5 mg (10 mg ml^{-1}) PLA crystals (×64). Stain: Haematoxylin & eosin.

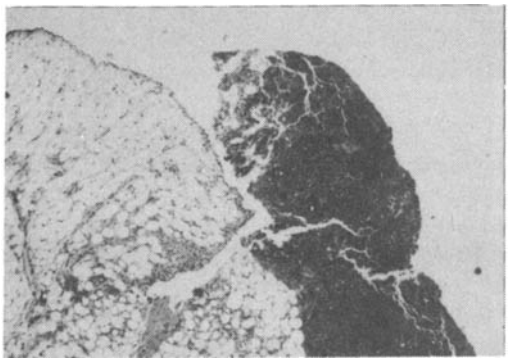

FIG. 3. Section through rabbit synovium 3 days after injection of 2·5 mg (10 mg ml^{-1}) PBCA crystals (×64). Stain: Haematoxylin & eosin.

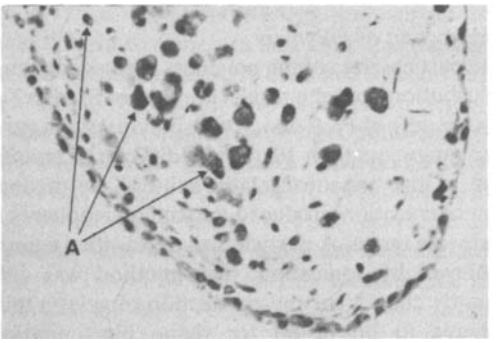

FIG. 5. Section through rabbit synovium 3 days after injection of 2·5 mg (10 mg ml^{-1}) PA microspheres (×630). A = PA microspheres within the synovium. Stain: Haematoxylin & eosin.

post-injection developed areas of avascular, necrotic tissue (Fig. 4).

PA microspheres were the most acceptable preparation to the joint tissues. There was little hyperplasia of the membrane and all underlying tissues were normal. PA microspheres could be detected in the phagocytic cells of the synovial membrane due to uptake of the eosin stain (Fig. 5). PA microspheres having a diameter of less than approximately 6 μm were readily phagocytosed by the synovial cells whereas those with a diameter greater than 6 μm were excluded. Slight hyperplasia of the membrane occurred where there was an accumulation of PA microspheres. The PA microspheres could still be readily discerned in the joint tissues at 7 days post-injection.

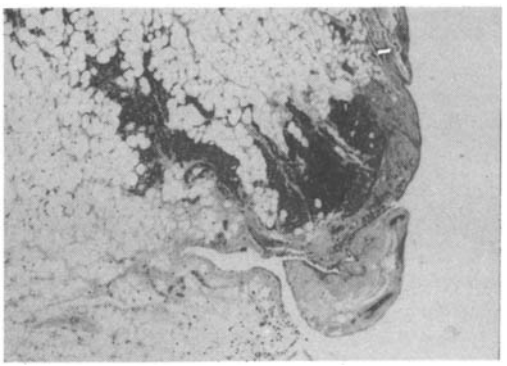

FIG. 4. Section through rabbit synovium 7 days after injection of 2·5 mg (10 mg ml^{-1}) PG microspheres (×160). Stain: Haematoxylin & eosin.

DISCUSSION

The feasibility of using a biodegradable, colloidal drug delivery system for intra-articular administration has been investigated. Because of rapid equilibration between the synovial fluid and plasma, clearance of soluble drugs from the joint results in the release of appreciable levels of the drug into the systemic circulation. Entrapment of a drug in a colloidal system which is phagocytosed by the synovial cells will delay this clearance as well as minimize the exposure of the cartilage to significant concentrations of the drug. This latter property will be of significant benefit with regard to corticosteroids which have been shown to produce marked deleterious effects on articular cartilage (Salter et al 1967). Ingrand (1973) suggested that large colloidal particles are cleared more slowly than small particles. However, Williams et al (1976) found little difference in the retention of two colloids of differing particle size. Benacereff et al (1975) observed that particles within the size range 0·0025 to 10 μm are readily phagocytosed. Our results show, however, that for maximal phagocytic uptake by the synovial cells the peak particle size should be less than 5 μm.

The suitability of a material for use as a drug-carrier is dependent on its tissue acceptability. Studies involving implants of PLA have indicated that it is a biodegradable, non-toxic material which has no inflammatory effect on surrounding tissues (Kulkarni et al 1966). Large microspheres (100 μm) of PLA have been produced as drug carriers for progesterone (Beck et al 1979). However stable microspheres of a size that is readily phagocytosed have not, so far been produced. Our studies have

shown that intra-articular administration of a particulate suspension of PLA results in an inflammatory response by the soft tissues of the joint. How much of this is due to the chemical nature of the material is unclear at this stage. It is well known that intra-articular administration of microcrystalline adrenocorticosteroid esters, such as hydrocortisone acetate, induces a reversible inflammatory response known as a 'post-injection flare' (McCarty & Hogan 1964). The inflammation that accompanies gout is recognized as being due to the presence of monosodium urate and calcium pyrophosphate crystals (McCarty & Hollander 1961). Injection of hydroxyapatite crystals into canine knee joints is also known to induce inflammation (Schumacher et al 1977). Such inflammatory lesions may be due to the release of enzymes (lysosomal and non-lysosomal) from synovial macrophages which occur following stimulation of these cells with monosodium urate crystals (McMillan et al 1981). We have also found that PLA polymers stimulate lysosomal enzyme release from macrophages, which may explain their inflammatory action in-vivo (Hunneyball & Smith, unpublished observations).

The polyalkylcyanoacrylates have been used as surgical adhesives for some years. The degradation rate of these polymers is a function of the alkyl chain length, the shorter methyl chain being degraded at a much faster rate than the butyl ester (Pani et al 1968). The tissue necrotic action of the methyl ester is well known (Woodward et al 1965) and is thought to be due to the rapid build up of degradation products, formaldehyde being the major one (Leonard et al 1966). The butyl ester with its slower rate of degradation is well tolerated in-vivo (Leonard 1970) although Mori et al (1967) in a study of cyanoacrylate surgical adhesives found little difference between the ethyl, propyl and butyl esters. Couvreur et al (1982) have investigated the potential of polybutylcyanoacrylate nanoparticles as a means of controlling the release of cytotoxic drugs. In-vitro toxicity studies showed that cellular damage was only induced at relatively high concentrations (1%) within the culture medium. After subcutaneous administration of these particles into mice however, no tissue necrosis or irritation was present.

In our studies a severe inflammatory response to the PBCA particulates was observed. Formaldehyde, a breakdown product of the cyanoacrylates and of known tissue toxicity (Hendrick & Lane 1975), is a likely cause of the inflammation. In-vitro studies on the toxicity of PBCA nanoparticles to rat hepatocytes showed that the magnitude of loss of cell viability was dependent on the concentration of formaldehyde produced by the degrading particles (Ratcliffe, Paterson, Wilson, Fry and Kreuter—unpublished data). The hydrophobic nature of PBCA resulted in aggregation of the particles despite the incorporation of a surfactant in the vehicle. This gave rise to a very localized response.

The inflammatory response produced as a result of the intra-articular administration of PG microspheres was much less severe than that of the PBCA. To date no adverse tissue reaction has been reported following systemic administration of gelatin microcapsules. Formaldehyde was used as the cross-linking agent for the gelatin and this binds strongly to gelatin, any excess being difficult to remove (Clark & Courts 1977). This residual formaldehyde is, therefore, the most likely cause of the inflammation observed. Unreacted formaldehyde may be removed from such particles by treatment with a sulphite (Oppenheim & Speiser 1978) which might decrease the tissue toxicity of gelatin microspheres.

The most acceptable polymer, of those tested, to the tissues of the knee joint was PA. The slight hyperplasia of the synovial membrane observed may have been due to the denaturation of the rabbit serum albumin with glutaraldehyde. Alternatively, this may represent a normal response to the particulate nature of the microspheres.

The limited nature of this response, together with the observed uptake of the small microspheres by the synovial cells implies that these microspheres should be suitable as an intra-articular drug delivery system.

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