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Albumin microspheres for intra-articular drug delivery: investigation of their retention in normal and arthritic knee joints of rabbits

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The retention of ^{131}I-labelled albumin microspheres and microsphere-entrapped [^{131}I]rose bengal was investigated in normal and experimentally arthritic knee joints of rabbits. Albumin microspheres were cleared slowly from the joint cavity and no significant difference was observed between normal and inflamed joints. Entrapment of rose bengal within albumin microspheres was found to delay the clearance of the drug from the joint when compared with a solution of rose bengal. In addition, the retention time for entrapped rose bengal was dependent on the degree of inflammation present.

Intra-articular administration of corticosteroids for the local treatment of rheumatoid arthritis was first advocated by Hollander et al (1951). However, due to the rapid clearance of the drug from the joint cavity, the duration of action is short-lived (Goetzl et al 1974) and high concentrations of the drug must be injected in the joint cavity to achieve a therapeutic effect (Hollander 1972). A significant increase in the efficacy of the drug has been attained by incorporation of steroids within liposomes (Dingle et al 1978). However, the anti-inflammatory effect of such a system could only be sustained for 3–4 days in rabbits (Shaw et al 1979) and 14 days in humans (De Silva et al 1979).

Incorporation of a drug within colloidal suspensions of biodegradable polymers may provide a more effective means of improving the retention of the drug within the joint cavity. In a previous study, polylactic acid, polybutylcyanoacrylate, polymerized gelatin and polymerized albumin were assessed for biocompatibility with synovial tissues (Ratcliffe et al 1984). Polymerized albumin microspheres were found to be best tolerated by the tissues and consequently in the present study the retention of albumin microspheres in both normal and inflamed knee joints has been investigated in rabbits. The retention of a model compound, rose bengal, entrapped within the microspheres has been studied to assess the potential of such systems as drug delivery devices. Rose bengal is a water-soluble compound which can be readily labelled with the gamma-emitting isotope iodine-131 and can be easily incorporated into the albumin microspheres due to its high protein affinity (Forker & Luxon 1983).

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MATERIALS AND METHODS

Iodination of albumin solution

Rabbit serum albumin was labelled with ^{131}I by modification of the method of Hunter & Greenwood (1962). 100 μL rabbit serum albumin (125 mg mL^{-1} in phosphate buffer, pH 7·4) and 10 μL chloramine T (200 mg mL^{-1} in phosphate buffer, pH 7·4) were added to 37 MBq Na^{131}I (Amersham, UK) and mixed gently for 5 min at room temperature (20 °C). 25 μL Sodium metabisulphite (60 mg mL^{-1} in phosphate buffer, pH 7·4) was added to quench the reaction. Separation of the labelled rabbit albumin from the reaction mixture was carried out by gel filtration on a 5 mL column of Sephadex G10 (Pharmacia) eluting with phosphate buffer (pH 7·4) containing 2% w/v ovalbumin. Fractions were collected after an elution volume of 1 mL, counted on a gamma counter and those corresponding to the labelled albumin retained.

The percentage incorporation of the label was determined by trichloroacetic acid precipitation of 5 μL iodinated albumin solution.

Preparation of albumin microspheres

Rabbit serum albumin microspheres (geometric mean = 3·5 μm, s.d. = ±1·74) were prepared by a method similar to that previously described (Ratcliffe et al 1984) by polymerization from a water-in-oil emulsion. A 12·5% solution of rabbit serum albumin (Fraction V Sigma, UK) in 0·07 M phosphate buffer (pH 7·6) was added to 25 mL olive oil and stirred at 2000 rev min^{-1} (Heidolph, Type RR1) for 5 min. Glutaraldehyde solution (350 μL, 50% v/v) (BDH) was added to cross-link the albumin and the emulsion stirred for a further 15 min. The micro-

spheres were collected by centrifugation at 2500g for 20 min and washed with light petroleum (b.p. 60–80 °C) followed by ethanol.

The microspheres were sized using a Coulter Counter (Model TA, Coulter Electronics) and examined microscopically using a scanning electron microscope (Jeol-35C). Incorporation of [^{131}I]albumin into the microspheres during preparation had no effect on the subsequent size distribution and the microspheres were smooth and spherical in appearance (Fig. 1a) but with a highly porous structure (Fig. 1b).

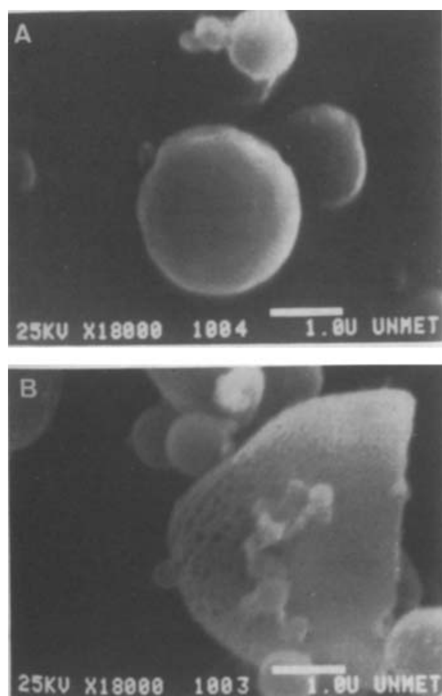

FIG. 1. Scanning electron micrographs (×18 000 magnification) of albumin microspheres showing: (a) spherical nature and (b) surface porosity.

Incorporation of rose bengal into albumin microspheres

Unlabelled rose bengal solution (0·2 mL) in 0·07 M phosphate buffer (pH 7·6, 50 mg mL^{-1}) was added to 37 MBq [^{131}I]rose bengal (Amersham, UK) which had been freeze-dried over 24 h (−75 °C, 100 millitorr vacuum). 25 mg Rabbit serum albumin was dissolved in the rose bengal (RB) solution and the microspheres prepared as described above. The final amount of RB incorporated into the microspheres was determined by analysis of the washings using spectrophotometry.

In-vitro release of rose bengal from albumin microspheres

Albumin microspheres, 25 mg, containing 5 mg [^{131}I]RB (19 kBq) were suspended in 200 mL 0·07 M phosphate buffer (pH 7·4) or human serum in a sealed, jacketed beaker at 37 °C. The suspension was stirred continuously at a constant rate using a magnetic stirrer. Aliquots of 1 mL were removed at various times up to 7 days. The samples were centrifuged at 3000 rev min^{-1} for 5 min and the amount of drug released from the microspheres determined by counting 0·5 mL supernatant in a gamma counter. All aliquots removed were replaced with 1 mL 0·07 M phosphate buffer (pH 7·4) or human serum.

Induction of experimental arthritis in the knee joints of rabbits

Mono-articular arthritis was induced in the knee joints of New Zealand White (NZW) rabbits (2·5–3·0 kg) by modification of the method of Dumonde & Glynn (1962) using ovalbumin (Sigma, UK) as antigen as described by Consden et al (1971). The contralateral knee was used as the control joint. Joint diameters of both the inflamed and control knees were measured daily using Vernier callipers. All experiments were carried out 4 days after the intra-articular injection of the antigen.

At the end of the experiment the infra-patellar folds containing the synovial membrane and submembranous adipose tissue were dissected from the joints, fixed in buffered formalin and embedded in paraffin wax. Sections (5 μm) were cut at two levels through each block, stained with haematoxylin and eosin and examined under a microscope. The presence of an inflammatory response similar to that observed in the acute phase of rheumatoid arthritis in man was confirmed.

In-vivo release of albumin microspheres from normal and arthritic rabbit joints

2·5 mg (10 mg mL^{-1} in 0·9% NaCl-saline) ^{131}I-labelled albumin microspheres (370 kBq) were injected into both normal and arthritic joint cavities of six New Zealand white rabbits. Clearance of activity from the joint was followed by gamma scintigraphy using an IGE Maxi Camera II coupled with a Link Systems computer (Hardy & Wilson 1981). Lateral images of the rabbit were recorded at various time intervals up to 14 days using a high energy, parallel collimator. A region of interest was defined around the joint and the radioactivity within this region

quantified correcting for radioactive decay and background activity.

To check the integrity of the radiolabel a control experiment was carried out using free $Na^{131}I$. 0·25 mL sodium iodide solution containing 925 kBq $Na^{131}I$ in saline was injected into the normal joint cavity of six rabbits and the rate of clearance determined as before.

At the end of the experimental period the rabbits were killed and various tissues dissected from the arthritic joint. The distribution of activity within these tissues was determined using a gamma counter (CG 4000, Intertechnique).

In-vivo release of free and albumin-entrapped rose bengal from normal and arthritic rabbit joints
2·5 mg albumin microspheres (10 mg mL^{-1} in saline) containing [^{131}I]RB (300 μg RB mg^{-1} albumin; specific activity 278 kBq mg^{-1} albumin) or 0·25 mL free [^{131}I]RB solution (1·8 MBq) were injected into normal and arthritic knee joints of six rabbits. Clearance of the activity from the joint with time was followed by gamma scintigraphy as before.

Blood samples were also taken from the marginal ear vein at various time intervals throughout the experiment and the activity present determined using a gamma counter.

RESULTS

Release of albumin microspheres from normal and arthritic joints
Clearance of the activity from the normal joint following injection of free $Na^{131}I$ solution was rapid, 14% (s.e.m. = ±1·6) remaining after 30 min (Fig. 2). The plateau observed after this time corresponds to circulating blood levels of $Na^{131}I$, which were cleared from the body at a much slower rate over 48 h.

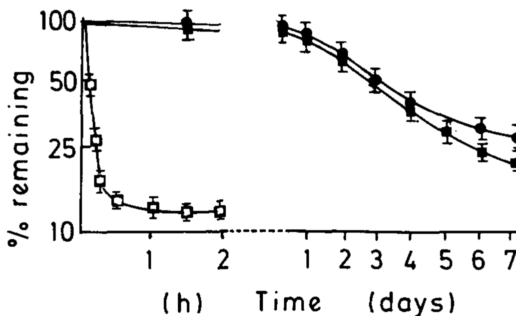

FIG. 2. Retention of [^{131}I]albumin microspheres in normal and arthritic rabbit knee joints as determined by gamma scintigraphy (n = 6, ±s.e.m.). □ $Na^{131}I$—normal joint, ■ [^{131}I]Albumin microspheres—normal joint. ● [^{131}I]-Albumin microspheres— arthritic joint.

Clearance of activity from the normal joint after injection of [^{131}I]albumin microspheres was slow compared with that of the free iodide, 50% of the dose remaining after 3 days.

Following the induction of arthritis in the rabbit knee joint, a 20–30% increase in joint diameter was observed 24 h following injection of the antigen into the joint cavity. The arthritic joint remained swollen throughout the experimental period. Histological examination at the end of the experiment confirmed the presence of pronounced inflammatory reaction in the arthritic joints.

There appeared to be no significant difference in the retention of [^{131}I]albumin microspheres in normal or arthritic joints (Fig. 2). Dissection of the tissues from the inflamed joint 10 days after injection showed that 80% (s.e.m. = ±6·6) of the remaining [^{131}I]albumin microspheres were found within the synovium, the remaining activity being associated mainly with the patella and menisci (Table 1).

Table 1. Per cent distribution of remaining [^{131}I]albumin microspheres in the arthritic knee joint 10 days post-injection (n = 6, ± s.e.m.).

Site	(%)
Ligamentum patella	2·0 ± 1·7
Patella	10·0 ± 2·5
Flexor digitorum longus	2·0 ± 1·8
Synovium	80·0 ± 6·6
Cartilage	1·5 ± 1·0
Menisci	8·5 ± 2·4
Cruciate ligaments	1·5 ± 1·2

Incorporation and in-vitro release of rose bengal from albumin microspheres
The percentage incorporation of RB into the albumin microspheres during preparation was 58% (s.e.m. = ±1·5). Subsequent release of the RB from the microspheres, when suspended in buffer, was slow, 66% (s.e.m. = ± 1·3) remaining after 7 days (Fig. 3). However, when the microspheres were suspended in serum there was a rapid initial phase of drug release followed by a slower second phase. 20% (s.e.m. = ± 2·9) of the RB remained incorporated in the microspheres after 6 days.

Retention of free rose bengal and albumin-entrapped rose bengal in normal and arthritic rabbit joints
Free [^{131}I]RB solution was cleared relatively slowly from normal and arthritic joints, with similar elimination profiles, 2% (s.e.m. = ± 0·5) being retained after 7 days (Fig. 4). Clearance of RB from the joint

cavity was delayed further by entrapment within albumin microspheres. The activity was cleared in a biphasic manner from both normal and inflamed joints. This consisted of rapid initial clearance during the first 48 h followed by a slower second phase of elimination. After 7 days, 25% (s.e.m. = ± 1·4) of the label remained within the inflamed joint and 9% (s.e.m. = ± 0·7) remained in the normal joint.

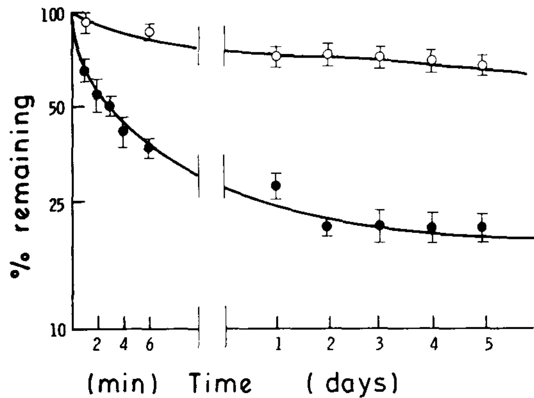

FIG. 3. In-vitro release of [^{131}I]rose bengal from albumin microspheres at 37 °C (n = 4, ± s.e.m.) in the presence of: ○ phosphate buffer pH 7·4, ● human serum.

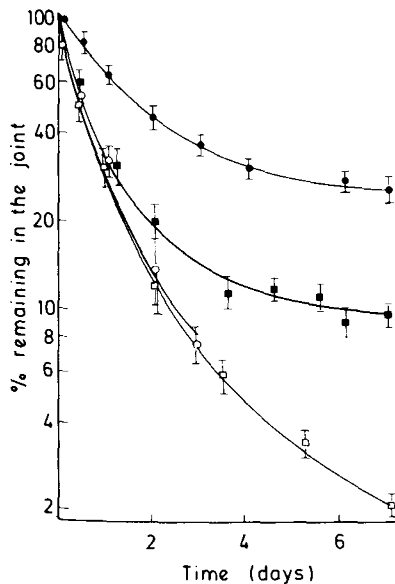

FIG. 4. Retention of free and albumin-entrapped [^{131}I]rose bengal from normal and arthritic rabbit knee joints as determined by gamma scintigraphy (n = 6, ±s.e.m.). □ Free rose bengal—normal joint. ○ Free rose bengal—arthritic joint. ■ Albumin-entrapped rose bengal—normal joint. ● Albumin-entrapped rose bengal—arthritic joint.

The blood activity-time profiles (Fig. 5) reflected the retention of the RB within the joint cavity. Thus [^{131}I]RB appeared in the blood soon after administration of the free drug into the joint. In contrast, entrapped RB administered into the arthritic joints reached maximum activity in the blood 1–2 days after injection. Analysis of the slopes after the times to peak, by covariance, showed no significant difference in the elimination of RB from the circulation following injection into normal and arthritic joints (variance ratio = 4, $P > 0·5$), whereas a significant difference was observed when the RB was entrapped within the microspheres (variance ratio = 36, $P < 0·001$).

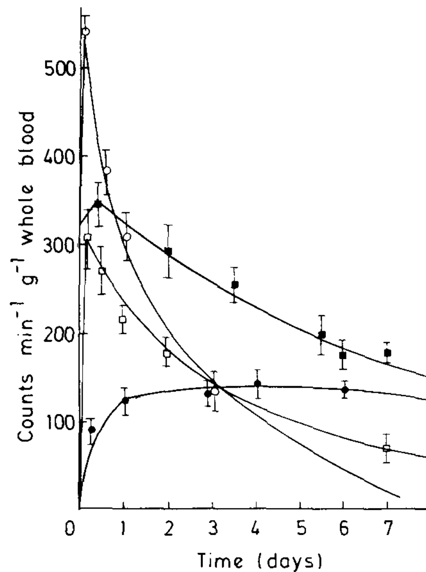

FIG. 5. Appearance of radioactivity in venous blood following intra-articular injection of: □ Free rose bengal—normal joint. ○ Free rose bengal—arthritic joint. ■ Albumin-entrapped rose bengal—normal joint. ● Albumin-entrapped rose bengal—arthritic joint.

DISCUSSION

A major limitation in the administration of soluble drugs by the intra-articular route is their rapid clearance into the systemic circulation (Wigginton et al 1980). Various particulate systems have been investigated with the aim of developing a system which is retained effectively within the joint. Webb et al (1969) showed that intra-articularly injected colloidal particles are taken up by both normal and inflamed synovium; however there was still some degree of extra-articular spread with these materials. Ingrand (1973) suggested that extra-articular spread was associated with particle size; the smaller the

particle the greater the leakage. Subsequently, Hnatowich et al (1978) showed that clearance of large macroaggregates of ^{165}dysprosium-labelled ferric oxide from the joint cavity was greatly reduced when compared to the smaller colloid, gold. However, Williams et al (1976) found that the retention of colloidal particles was independent of size. Furthermore, other workers have shown that other factors such as physical form and degree of inflammation may also influence the clearance rate (Goode & Howey 1973; Gumpel 1978; Kyle et al 1983).

The present study showed that there was a slow leakage of radioactivity from the joint cavity following injection of [^{131}I]albumin microspheres which was independent of the inflammation present. However, using the technique of gamma scintigraphy it was not possible to discern whether whole microspheres were leaving the joint cavity or whether the microspheres had been degraded intracellularly, with the loss of radioactivity corresponding to the loss of small peptides. In this regard, iodination of the albumin molecules may increase the denaturation of the protein thereby affecting subsequent degradation of the microspheres and clearance kinetics (Greenwood & Hunter 1963; McConahey & Dixon 1966). In addition, Noble et al (1983) found a greater leakage of activity from the joint cavity following administration of biodegradable colloids when compared with inert colloidal particles which could be attributed to the degradation of the particles by lysosomal enzymes in the joint.

Materials injected into the joint cavity are absorbed into the circulation either via the blood capillaries that supply the synovium or via the lymphatics or by a combination of the two (Levick 1980). The contribution of lymphatic drainage is unknown but if the joint is immobilized, for example by splinting, the lymphatic drainage is known to be minimal (Trimbell-Fischer 1923) and the extra-articular spread reduced (Oka et al 1971). Furthermore, Meachim & Brooke (1983), using cobalt-chromium-molybdenum particles, confirmed that lymphatic transport of colloidal particles from the joint occurs.

The studies on the distribution of the radiolabelled albumin microspheres in the joint reported here confirmed our original microscopic observations (Ratcliffe et al 1984) that the microspheres appear to be taken up rapidly by the synovium. The lack of radioactivity in other tissues which are in contact with the synovial fluid, and hence with the injected microspheres, supports the conclusion that the microspheres are taken up by phagocytic cells within the synovium. This is further supported by the observation that albumin microspheres are rapidly phagocytosed by macrophages in-vitro (unpublished observations).

The release of rose bengal from the microspheres was relatively slow, particularly in relation to other molecules investigated in this system (Tomlinson & Burger 1985). This is due to its high affinity for proteins (Forker & Luxon 1983) which probably also accounts for the slow clearance of free rose bengal from the joint cavity as compared with the rapid elimination of free sodium iodide. Incorporation of rose bengal within the microspheres decreased considerably its rate of elimination from the joint in comparison with the free compound. Unlike the radiolabelled microspheres themselves, microsphere-entrapped rose bengal was retained to a greater extent in the inflamed than in the normal joint. There are several possible explanations for this including the increased number of phagocytic cells within the inflamed joint as a result of synovial cell proliferation (Henderson et al 1982), and the deposition of proteins such as fibrin onto which the rose bengal may be adsorbed. Obviously, during the period under investigation, there will be considerable diffusion of rose bengal out of the microspheres which complicates the interpretation of this difference between normal and arthritic joints.

Nevertheless, this study has shown that albumin microspheres are retained within both normal and arthritic joints and that this type of system can be used to retain molecules such as rose bengal within the joint for a prolonged period of time. However, this degree of drug retention may not be easily achievable with molecules that do not have a high affinity for proteins.

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