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Potential use of albumin microspheres as a drug delivery system. II. In vivo deposition and release of steroids

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Summary

The in vivo drug release characteristics of heat-stabilized albumin microspheres containing corticosteroid drugs were investigated. The drug–plasma levels were determined following intra-articular and intramuscular administration of the preparations to New Zealand White rabbits. The microsphere preparations showed sustained release characteristics in vivo compared to control microcrystalline suspensions of the drugs. The relative $t_{1/2}$ values for drug release in vivo were related to the $t_{1/2}$ values for drug release in vitro. The microspheres used in this study were of relatively large particle diameter (23 μm) and were not phagocytosed by mouse peritoneal macrophages in culture or by the macrophages within the synovial cavity.

Introduction

Direct administration of corticosteroidal drugs into the affected joints of sufferers of rheumatoid arthritis has been advocated (Hollander et al., 1951), as a means of reducing the adverse side-effects associated with this type of drug therapy. However, multiple dosing of steroid drugs, directly into the joints has a deleterious effect on the articular cartilage, (Chandler, 1959; Salter et al., 1967) and a means of reducing the number of doses has therefore been sought. The aim of this study was to prepare albumin microspheres containing corticosteroids which would have an acceptable release profile for the drug over a period of weeks or months when injected into the synovial cavity.

The in vitro release of prednisolone from albumin microspheres stabilized by heat or chemical denaturation has been reported previously (Burgess et al., 1987; Davis et al., 1987). Chemical denaturation of albumin using glutaraldehyde did not have a marked effect on drug release from the microspheres. Heat denaturation, on the other hand, significantly sustained the in vitro release rate of corticosteroids from the microspheres, compared to microcrystalline drug suspensions. The greater the extent of heat denaturation of the albumin, the slower the drug release rate. Heat denaturation was therefore chosen as the method of stabilization for in vivo studies.

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Prednisolone and triamcinolone were selected for study. Triamcinolone has a comparable effect to prednisolone at a similar dosage, but has the advantages of a longer plasma half-life and a slower clearance rate from the joint (Dixon et al., 1972). For these reasons microcrystalline suspensions of triamcinolone are used clinically for intra-articular therapy (Reynolds, 1982). In order to compare the results with other studies involving corticosteroid preparations reported in the literature two injection routes were studied: intra-articular and intramuscular.

An in vitro investigation of the uptake of microspheres by cultured mouse peritoneal macrophage is also reported. This cell culture model has been used previously with success to investigate the uptake of colloidal particles by macrophages (Illum et al., 1986). Microspheres are known to be phagocytosed by the macrophages residing in the epithelium lining the joint synovium (Ratcliffe, 1984). This may influence drug release from the microspheres, in that the drug will have to diffuse from the macrophage but also the enzymes present within the lysosomal compartment of the macrophage may speed up degradation of the microspheres and hence drug release. However, since relatively large microspheres were used in the present study, namely 23 μm diameter, it is unlikely that these would be taken up by synovial macrophages. According to the work of Ratcliffe (1984) particles greater than 6 μm are not taken up in vivo by synovial macrophages. In vivo uptake of the microspheres by macrophages has been determined by post-mortem examination.

Materials and Methods

Rabbit serum albumin (RSA) fraction V, highly refined olive oil, prednisolone, triamcinolone, Tween 80, sodium heparinate, benzylpenicillin, and streptomycin sulphate were all obtained from Sigma, U.K. Polycarbonate membrane filters, 0.2 and 0.8 μm pore diameter were obtained from Nucleopore, Diethylether, potassium phosphate, and sodium hydroxide were of AnalaR grade, from BDH Chemicals. [^{3}H]Prednisolone and [^{3}H]triamcinolone were obtained from Amersham International U.K. Lumagel scintillation cocktail was obtained from Lumac, Schaesberg, The Netherlands. Tissue culture medium E199 with Earle's salts was obtained from Flow Laboratories, U.K.

Preparation of tritiated drug and drug–microsphere suspensions

The tritiated drug was diluted by mixing with cold drug dissolved in acetone and the mixture was rotary evaporated to dryness. The resultant powder was suspended in a sterile solution of normal saline containing 0.1% Tween 80. The final drug suspensions contained 0.25 mg of drug and 100 μCi of tritium (prednisolone) or 167 μCi of tritium (triamcinolone) in each 0.125 ml.

Microspheres containing radiolabelled drug were prepared by suspending the diluted, tritiated drug powder in an aqueous albumin solution (20% w/v). This suspension was emulsified in olive oil and microspheres with a geometric mean particle diameter of 23 μm and a standard deviation of 0.67 μm were produced as previously described (Burgess et al., 1987). The microspheres were stabilized to varying extents using a hot air oven. Prior to injection, the microspheres were suspended in a sterile solution of normal saline containing 0.1% Tween 80. Each 0.125 ml of suspension contained 0.25 mg of microspheres and 100 μCi of tritium (prednisolone) or 133 μCi of tritium (triamcinolone). A drug to microsphere weight ratio of 1 : 5 was used.

In vitro drug release

Microspheres were prepared as above. The release of cold drug from the microspheres 23 μm mean diameter, into phosphate buffer (pH 7.0) at 37 °C, was determined under sink conditions by UV analysis correcting for RSA absorbance. Approximately 20 mg of microscopheres were added with stirring to 350 ml of phosphate buffer (pH 7.0) in a water-jacketed beaker at 37 °C.

Animal experiments

Male New Zealand White (NZW) rabbits, weighing 3–5 kg, were used. The rabbits were divided into groups of 12 and each group was

injected either intramuscularly or intra-articularly with microspheres containing prednisolone or triamcinolone. Control groups were injected by the same routes, with microcrystalline drug suspensions. 0.125 ml aliquots of suspension were injected into either the intra-articular space of the left rear knee or into the left rear thigh muscle (tensor fascia lata) of each rabbit. Blood samples were withdrawn from the marginal ear vein at approximately 30 min intervals from t_0 to $t = 4$ h, and at $t = 6$, 9, 12 and 24 h. Thereafter samples were taken at daily intervals until the tritium content, as measured by scintillation counting, reached background levels. 2 ml blood samples were taken from each rabbit and centrifuged immediately for 5 min. 0.5 ml of the resultant plasma was added to 10 ml of scintillation cocktail (Lumagel), in duplicate. The plasma was assayed for tritium content using an Intertechnique, PG 4000 scintillation counter (Kontron, U.K.).

The plasma drug levels, for each rabbit as measured in decays per minute (dpm) were corrected for dosage. The results for all the rabbits in the same group, which had received the same treatment, were pooled and the mean dpm values and standard deviations were calculated.

Non-stimulated mouse peritoneal macrophages

The method reported by Illum et al. (1986) was used. Strain MF1 mice, weighing 20–25 g, were used freshly killed by CO_2 asphyxiation. The lavage medium consisted of 10 ml tissue culture Medium El99 concentrate, 10 ml swine serum, 2.5 ml sodium bicarbonate 7.5%, 0.1 ml crystamycin, 6 mg heparin, and 77.4 ml sterile water. The viability of the macrophages was tested by exclusion of Tryptan blue and found to be at the order of 95%. The macrophage suspension was adjusted to a final cell count of 1.0×10^6 cells/ml using a Coulter Counter (model TAII) to determine the counts. 2.5 ml of cell culture medium containing the appropriate microspheres (5 particles per macrophage) was added to each plate and the plates were incubated for 5, 10, 20, 30 min, 1, 2, 3, 6, 9 and 12 h. The media was then removed from the plates, the cells washed twice with sterile phosphate-buffered saline, fixed with methanol for 5 min, and stained with Geimsa (1 : 10) for 3 h. The plates were washed with water and left to dry before counting the number of particles phagocytosed using an optical microscope. A total of 100 macrophage cells were counted. These experiments were done in triplicate.

Results and Discussion

In vivo drug release studies

The microsphere–drug preparations were stabilized to different extents in a hot air oven. The maximum temperature which prednisolone would withstand without a significant level of degradation was found to be 160 °C. 4% w/w of the prednisolone decomposed under these conditions. Triamcinolone would withstand 170 °C for 48 h, with 2.5% w/w decomposition. 150 °C was selected as a suitable stabilization temperature for both drugs. Two prednisolone microsphere preparations were studied: preparation A, which had been stabilized for 10 h at 150 °C and had an in vitro half-life ($t_{1/2}$) for drug release of 0.9 h; and preparation B which had been stabilized for 22 h at 150 °C and had an in vitro $t_{1/2}$ for drug release of 2 h. Two triamcinolone microspheres preparations were studied: preparation C, which had been stabilized for 12 h at 150 °C and had an in vitro $t_{1/2}$ for drug release of 1.0 h; and preparation D, which had been stabilized for 40 h at 150 °C and had an in vitro $t_{1/2}$ for drug release of 9.2 h.

The plasma radioactivity/time profiles following intra-articular administration of these different preparations, are shown in Figs. 1 and 2 for prednisolone and triamcinolone, respectively, and following intramuscular injection of triamcinolone in Fig. 3. Absorption of drug from the injection sites into the plasma was determined as a percentage of the total dose administered from the plasma radioactivity time profiles and the radioactivity remaining at the injection sites (Figs. 4, 5 and 6). The percentage drug absorbed from the injection site into the general circulation approximated 91% for the microsphere preparations compared to 96% for the suspensions. This difference may be a result of tightly bound drug remaining trapped within the microspheres after termination of the experiment. All of the prepara-

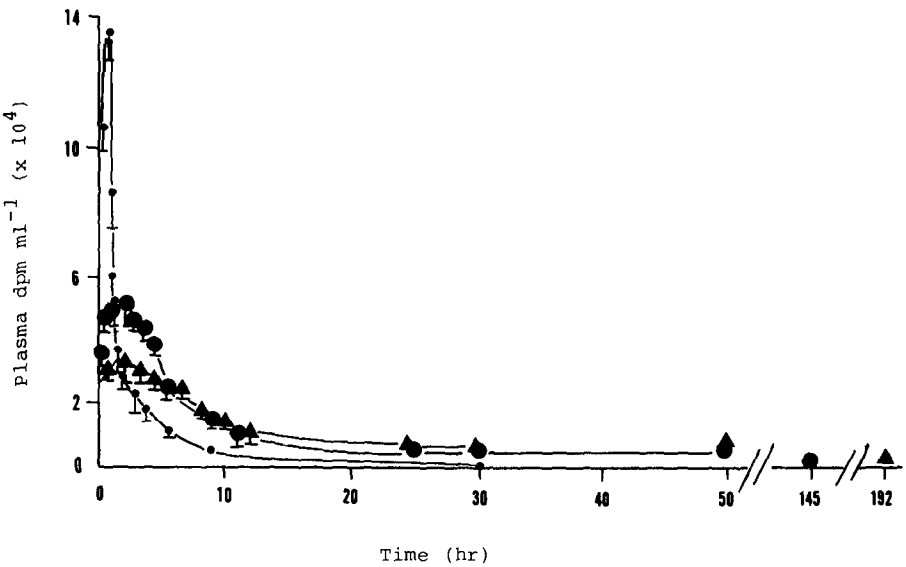

Fig. 1. Plasma radioactivity–time profiles following intra-articular injection of [^{3}H]prednisolone into NZW rabbits. Key: ·, suspension; ●, microsphere – preparation A, stabilized for 10 h at 150 °C; ▲, microsphere – preparation B, stabilized for 22 h at 150 °C.

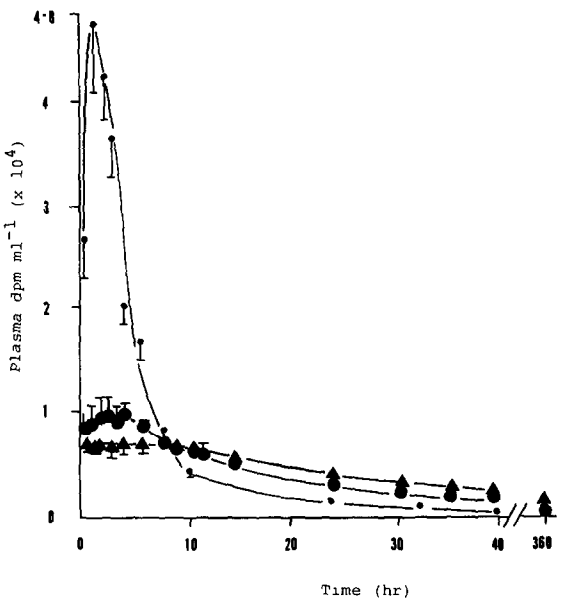

Fig. 2. Plasma radioactivity–time profiles following intra-articular injection of [^{3}H]-triamcinolone into NZW rabbits. Key: ·, Suspension; ●, microsphere – preparation C, stabilized for 12 h at 150 °C; ▲, microsphere – preparation D, stabilized for 40 h at 150 °C.

tions show biphasic first-order kinetics. The initial absorption phase is dependent on the formulation (drug release from the formulation). The second phase is independent of the formulation. The absorption rate of the prednisolone suspension ($t_{1/2}$, 5.0 h) following intra-articular injection was slightly faster than for the triamcinolone suspension ($t_{1/2}$ 8.1 h) which is in agreement with the data of Dixon et al. (1972), and Bird et al. (1979). Due to the lipophilic nature of triamcinolone it was absorbed more slowly from the intra-muscular site ($t_{1/2}$ 14.5 h) than from the intra-articular site. The absorption rates are dependent on both the sites of injection and the drug preparation. The drug suspensions have the fastest rates and the more highly stabilized microsphere preparations the slowest (Figs. 1, 2 and 3).

Assuming that the absorption and elimination of the drug are independent of the dosage form administered, relative in vivo $t_{1/2}$ values ($t_{1/2rel}$) for drug release from the microspheres can be calculated (Pollack et al., 1984). The $t_{1/2}$ values for the drug suspensions were subtracted from the $t_{1/2}$ values of the microsphere preparations to give

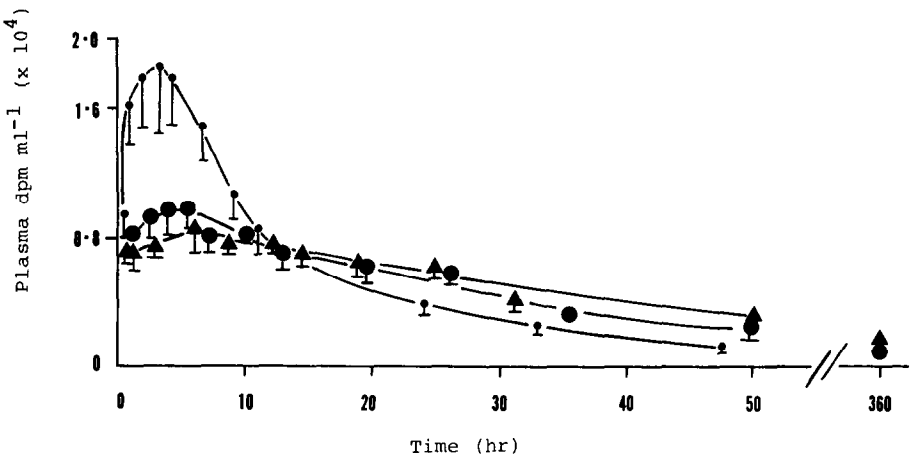

Fig. 3. Plasma radioactivity–time profiles following intramuscular injection of [^{3}H]-triamcinolone into NZW rabbits. Key: Suspension; ●, microsphere – preparation C, stabilized for 12 h at 150 °C; ▲, microsphere – preparation D, stabilized for 40 h at 150 °C.

$t_{1/2rel}$ values (Tables 1 and 2). The in vitro $t_{1/2}$ values obtained for the release of drug from these microspheres into pH 7.0 phosphate buffer are also shown in Tables 1 and 2. The in vitro release rates are much faster but are proportionally related to the in vivo release rates. The $t_{1/2rel}$ values for prednisolone administered by the intra-articular route are related to the $t_{1/2}$ in vitro values by a factor of 5.6 for both preparation A and B. The $t_{1/2\ rel}$ values for triamcinolone are related to the $t_{1/2}$ in vitro values by factors of 6.4 for preparation C and 5.9 for preparation D administered by the intra-articular route, and by factors of 4.2 for preparation C and 3.6 for preparation D administered by the intramuscular route. The differences in the $t_{1/2rel}$ in vivo and the $t_{1/2}$ in vitro release

TABLE 1

The effect of formulation on the half-life values for prednisolone release from microspheres: in vitro into pH 7.0 buffer at 37 °C; and in vivo following intra-articular injection into NZW rabbits

Formulation	$t_{1/2}$ in vitro (h)	$t_{1/2}$ in vivo (h)	$t_{1/2rel}$ (h) *
Suspension	0.05	5.0	
Microsphere preparation A, stabilized for 10 h at 150 °C	0.9	10.0	5.0
Microsphere preparation B, stabilized for 22 h at 150 °C	2.0	16.25	11.25

* $t_{1/2rel} = t_{1/2}$(Suspension) − $t_{1/2}$(Microsphere preparation)

TABLE 2

The effect of formulation on the half-life values for triamcinolone microspheres: in vitro into pH 7.0 buffer at 37 °C; and in vivo following intra-articular and intramuscular injection into NZW rabbits

Formulation	$t_{1/2}$ in vitro (h)	$t_{1/2}$ in vivo (h)	$t_{1/2rel}$ (h) *
(a) Intra-articular			
Suspension	0.1	8.1	
Microsphere preparation C, stabilized for 12 h at 150 °C	1.0	14.5	6.4
Microsphere preparation D, stabilized for 40 h at 150 °C	9.2	62.5	54.4
(b) Intramuscular			
Suspension	0.1	14.5	
Microsphere preparation C, stabilized for 12 h at 150 °C	1.0	18.75	4.25
Microsphere preparation D, stabilized for 40 h at 150 °C	9.2	47.5	33.0

* $t_{1/2rel} = t_{1/2}$(Suspension) − $t_{1/2}$(Microsphere preparation)

rates are due to the different release conditions. The drug release rates in vitro were determined under sink conditions into pH 7.0 phosphate buffer with constant stirring. The in vivo release rates are expected to be slower as the drug is released from the microspheres into either the synovial fluid, or into the fatty muscle tissue and will be dependent on degradation of the microspheres.

The results show that microsphere encapsulation in heat-stabilized albumin microspheres significantly retards the in vivo release of both prednisolone and triamcinolone and hence retards absorption from the injection site. The more stabilized the microsphere preparation the slower the in vivo release rate. Triamcinolone is the more attractive drug for this type of slow release therapy since the plasma levels are sustained for 15 days compared to 8 days for prednisolone. These results are in agreement with the results of other authors. Morimoto et al. (1980) and Sugibayashi et al. (1979) have obtained sustained release profiles of 5-fluorouracil over a period of one week from heat-stabilized microspheres following intraperitoneal injection into mice with Ehrlich ascites carcinoma. On injection of DPPC liposomes into NZW rabbits, by the intramuscular route, Arrowsmith et al. (1984) achieved sustained release of [^{3}H]cortisone hexadecanoate over a period of 14 days.

Macrophage tissue culture and in vivo deposition studies

An investigation was carried out into the maximum size of particles which the mouse peritoneal macrophage culture cells were able to phagocytose. Microspheres with mean diameters in the size range 1–36 μm were studied. Microspheres with mean diameters below 10 μm were all phagocytosed within 20 min of administration. 12 μm particles were all phagocytosed within 60 min of administration. 13 μm, 20 μm, 23 μm, and 36 μm particles were not ingested. Ratcliffe (1984) has reported that microspheres larger then 6 μm are not phagocytosed by synovial macrophages in vivo. This in vitro study shows that microspheres up to a mean particle diameter of 12 μm are phagocytosed by cultured mouse peritoneal macrophages. It can be concluded that the microspheres used in this study (23 μm, mean diameter) are too large for uptake by the synovial macrophage cells.

There is a concern that microspheres of this size might remain freely dispersed in the synovial fluid, thereby causing irritation by rubbing the joint surfaces. The in vivo deposition of the albumin microspheres was therefore studied. NZW rabbits were sacrificed 24 h to one month after intra-articular injection of a suspension containing 0.25 mg of highly stabilized microspheres (23 μm diameter) in normal saline containing 0.1% Tween 80. These highly stabilized microspheres were prepared by heating microspheres prepared as described in the methods section at 190 °C for 24 h. They were orange/red in color when viewed by light microscopy, and could be easily seen in the tissue. Being highly stabilized, they did not biodegrade during the course of the study. The synovial tissue and fluid were excised from both rear knees, post-mortem. The right rear knees were used as controls. No microspheres were found free in the synovial membrane either singly or in aggregates.

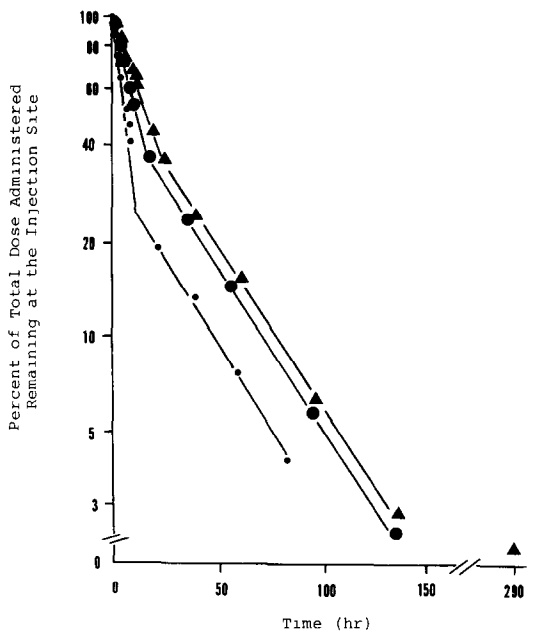

Fig. 4. The percentage of the total dose administered remaining at the injection site, following intra-articular injection of [^{3}H]prednisolone into NZW rabbits (logarithmic plot). Key: as in Fig. 1.

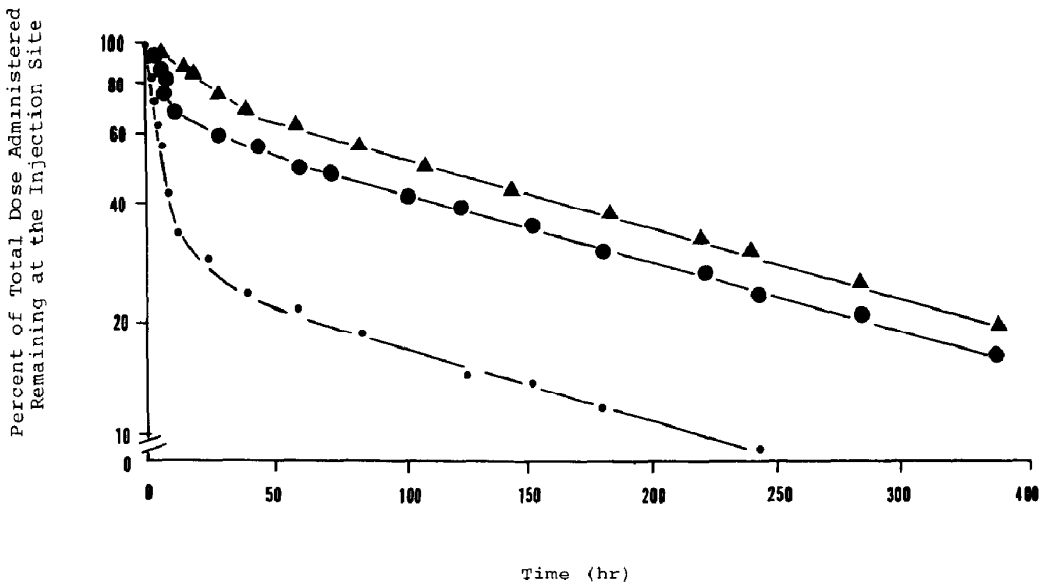

Fig. 5. The percentage of the total dose administered remaining at the injection site, following intra-articular injection of [^{3}H]triamcinolone into NZW rabbits (logarithmic plot). Key: as in Fig. 2.

The size of these aggregates was up to approximately 200 μm and were therefore too large for ingestion by a single macrophage. The microspheres may well have been surrounded by groups of macrophage cells or by adhering to the synovial membrane mediated by the adsorption of compo-

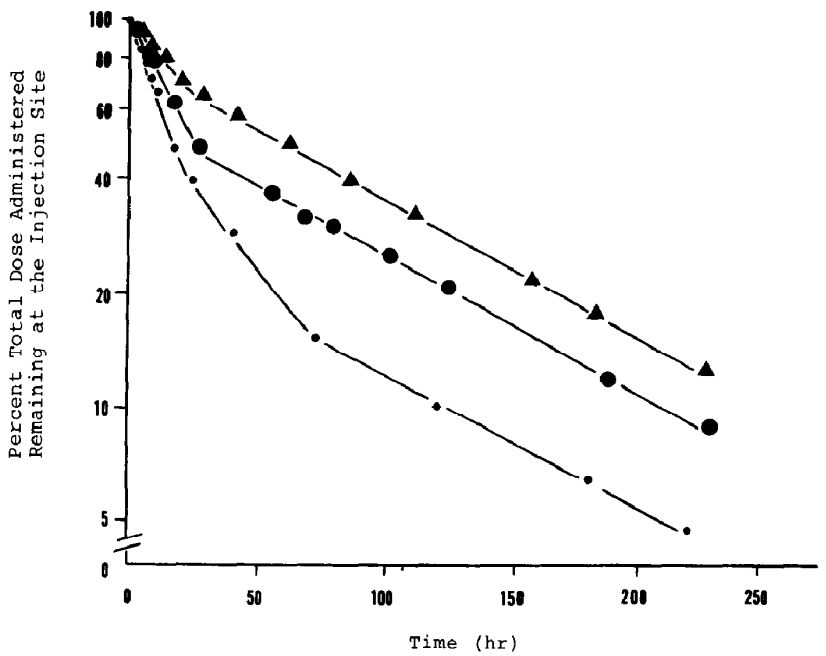

Fig. 6. The percentage of the total dose administered remaining at the injection site, following intramuscular injection of [^{3}H]triamcinolone into NZW rabbits (logarithmic plot). Key: as in Fig. 3.

nents from the synovial fluid. Hyaluronic acid, a major component of synovial fluid, is known to form strong viscoelastic films at interfaces (Kerr and Warburton, 1985) and may form a tight film around the microspheres thereby encapsulating and sticking them to the synovial membrane.

Conclusions

Albumin microspheres, 23 μm mean diameter, have been used successfully to sustain the release of prednisolone and triamcinolone when administered by intra-articular and intramuscular injection into rabbits and may thus have a possible clinical use as a sustained release drug delivery system. The relative in vivo $t_{1/2}$ values for drug release from the microspheres were directly related to the in vitro $t_{1/2}$ values. In addition, these relatively large microspheres were not taken up by macrophage cells either in vitro in a mouse peritoneal tissue culture or in vivo in the synovium of rabbits. The microspheres were not dispersed freely in the synovial fluid, but were associated with the synovial membrane.

Acknowledgements

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