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Targeting of microspheres to sites of inflammation

L. Illum, J. Wright and S.S. Davis

Department of Pharmaceutical Sciences, School of Pharmacy, University of Nottingham, Nottingham (U.K.)

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Summary

By coating small colloidal carriers with the block copolymer, Poloxamine 908, it has been possible to avoid the normal deposition of these particles in the liver and spleen and to obtain a considerable deposition of these otherwise circulating particles in induced sites of inflammation in thigh muscles of rabbits.

Introduction

Colloidal particles in the form of microspheres, emulsions and liposomes are being actively considered as potential systems for the site-specific delivery of drugs following intravenous administration (Gregoriadis, 1977). A major obstacle to targeting to sites inside and possibly outside the vascular compartment is the recognition and capture of particles by the host's defense system; namely the reticuloendothelial cells residing in the liver and to a lesser extent, the spleen (Poste and Kirsh, 1983). We have shown previously that intravenously administered microspheres and emulsions can be directed away from their normal site of capture in the liver, by means of polymeric coatings (Illum and Davis, 1987; Illum et al., 1987a). These prevent (or decrease) the uptake of plasma proteins (opsonins) onto particles and also prevent their interaction with the cells of the reticuloendothelial system (RES) by a steric repulsion (excluded volume) effect. In particular, polyoxypropylene–polyoxyethylene block co-polymers, comprising the poloxamer and poloxamine series, have been used to target particles to the bone marrow or to maintain particles in the general circulation (Illum et al., 1987a; Illum and Davis, 1987). This second option provides opportunities for the intravascular delivery of drugs such as thrombolytics, anti-infectives and anti-leukemics. In addition, the freely circulating colloidal carriers should have the ability to escape to extravascular sites in regions of inflammation because in such areas the normal barrier function of the endothelial lining of blood capillaries and the underlying basal membrane are often disrupted and the permeability thereof increased due to inflammatory oedema (Fuchs, 1977; Gedigk and Helpap, 1984). Mizushima et al. (1982), have attempted to exploit this concept when delivering anti-inflammatory agents using emulsion particles. However, the quantity of drug reaching an inflammation site (Carageenan-induced oedema in

Correspondence: L. Illum, Department of Pharmaceutical Sciences, School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

rat footpad) was very small (less than 0.5% of administered dose) and the majority of the drug was probably directed inadvertently to the cells of the RES in the liver and spleen. In a similar manner, colloidal systems in the form of albumin particles (less than 100 nm in diameter) have been used for diagnostic purposes in order to detect sites of inflammation (De Schrijver, 1986; De Schrijver et al., 1987). Again, only a small fraction of the particles (about 0.1%) accumulated in the inflamed tissues. An alternative procedure using indium-111-labelled neutrophils (Bitar et al., 1986) provided an accumulation of about 9.8% of the dose in subcutaneously implanted abscesses in rabbits 24 h after injection. Similarly, Ogihara et al. (1986) found about 6% of gallium-67-labelled liposomes to accumulate in subcutaneously implanted Yoshida sarcomas. It was suggested that the accumulation probably was due to structural defects in the endothelium and basement membrane in the microcirculation. We have reasoned that our particles, coated with the polymer, Poloxamine 908, and thereby circulating in the blood (Illum et al., 1987a) should provide an alternative strategy for the targeting of drugs and diagnostic agents to sites of inflammation.

Materials and Methods

A model test system in the form of polystyrene particles (80 nm in diameter (PCS 'Z'-average) (Douglas et al., 1984)) were labelled with iodine-131 using the method of Huh et al. (1974), as described previously (Illum et al., 1982). The particles were coated with poloxamine 908 (polyoxyethylene–polyoxypropylene co-polymer condensed on ethylenediamine) (BASF) by a process of physical adsorption resulting in an adsorbed layer thickness of about 13.4 nm as described earlier (Illum et al., 1987b). Chronic inflammation was induced in the left flank of New Zealand white rabbits ($n = 5$) (av. wt. 3.5 kg) using an intramuscular injection of 0.5 ml of a 1.5% solution of carageenan (Sigma). This method for the induction of inflammation has been described by DiRosa, (1972) and Ogihara et al. (1986). Seventy-two hours later the labelled polystyrene particles were injected via the marginal ear vein. Three rabbits received 0.6 ml of a suspension of coated particles (2.1 MBq) and two rabbits (controls) were given 0.3 ml (2.0 MBq) of uncoated particles. In all cases the animals received a total dose of about 10^{13} particles. The distribution of the labelled particles was followed by γ-scintigraphy (Gammascope, General Electric) for up to 24 h monitoring levels of activity in the whole animal and at the site of inflammation, taking anterior and lateral views. The data were stored in a computer and subsequently analysed by creating regions of interest around the whole animal, the injection site and contralateral site.

Results and Discussion

Some representative scintiphotos (24 h after injection) are shown in Fig. 1 that demonstrate the difference in particle deposition in animals with inflammation, treated with uncoated and coated particle systems, respectively. As previously described elsewhere (Illum et al., 1987a), non-coated particles were recognised as foreign and were removed rapidly by elements of the RES, particularly the Kupffer cells of the liver and the macro-

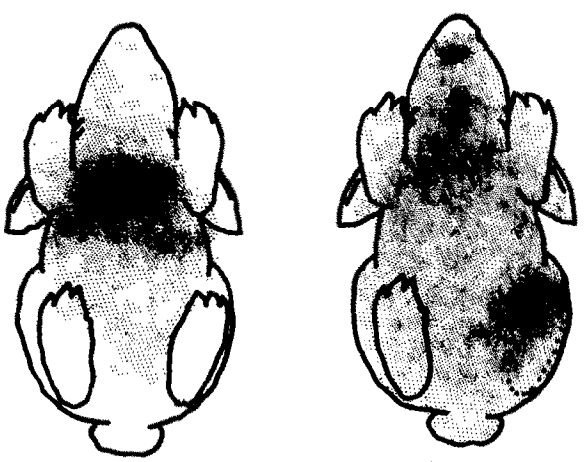

Fig. 1. γ-Scintigraphs (dorsal views) of rabbits with induced inflammation in the left thigh muscles 24 h after injection of non-coated polystyrene particles (A) and polystyrene particles coated with Poloxamine 908 (B).

TABLE 1

The accumulation of polystyrene microspheres non-coated or coated with Poloxamine 908 at the site of inflammation 24 h after injection

	Activity at site of inflammation % of total activity (±S.E.M.)
Microspheres	0.95 (±0.25) ($n = 2$)
Microspheres–Poloxamine 908	12.5 (±2.2) ($n = 3$)

phages of the spleen (Fig. 1A). Poloxamine 908-coated particles were, on the other hand, for the main part retained in the general circulation as would be expected from results in earlier work (Illum et al., 1987a) where there was no inflammation site present. However, a high degree concentrated at the site of inflammation was also found as shown by Fig. 1B.

In terms of percentage of the administered radioactivity, more than 10% of the dose reached the inflammation site for the coated particles as compared to less than 1% for the uncoated particles (Table 1). The values presented have been corrected for background and circulating activity. The particles could be first demonstrated in the inflammation site at 2 h after injection rising to a maximum at 24 h (Fig. 2).

The mechanism of selective uptake is as yet unclear. We have found that in the blood the Poloxamine 908-coated particles are not associated with any subset of blood cells. Ninety-nine percent of the activity (iodine-131) was found in

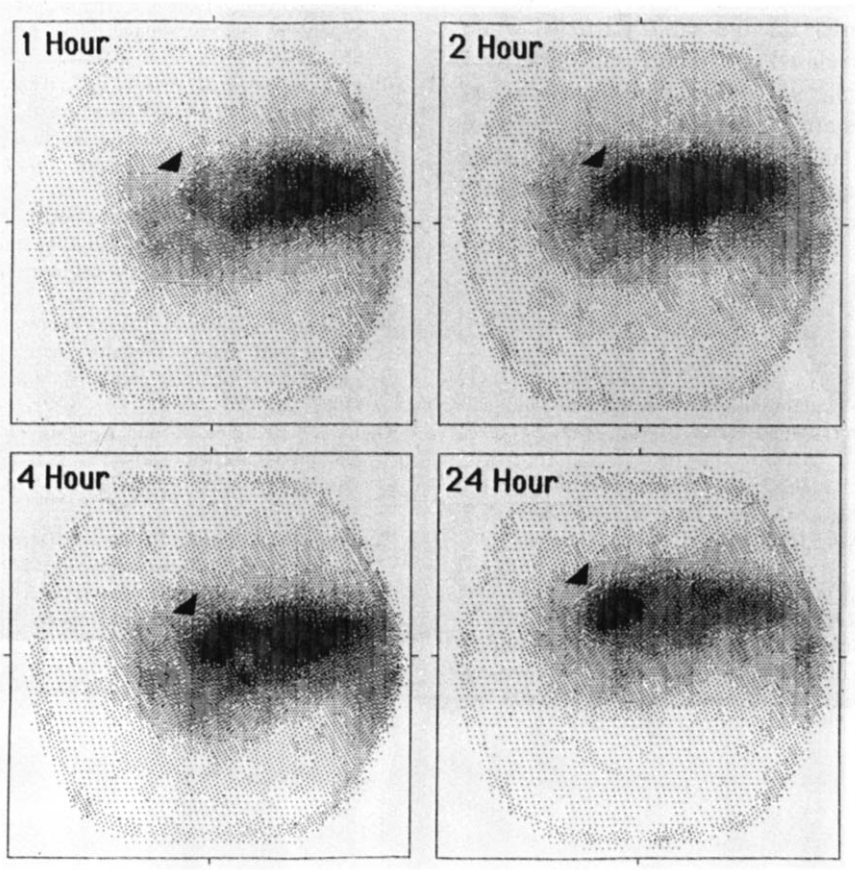

Fig. 2. γ-Scintigraphs (side views) of rabbits with induced inflammation in the left thigh muscle showing the accumulation of site of inflammation of polystyrene particles coated with Poloxamine 908 at different times after injection.

the plasma after separation by centrifugation of a blood sample taken 1 h after administration of the coated particles to rabbits without inflammatory sites. The particles may reach the inflammation site simply because the endothelium and the basal membrane in the inflamed region are disrupted and thus extravasation is possible. However, this would be expected to provide a level of activity in the tissue similar to that in the circulation and not a higher selective uptake. Therefore, the possibility exists that the particles are actively taken to the inflammation site by, for example, diapedesing poly-morphonuclear leucocytes (Fuchs, 1977; Schoefl, 1964). If this is the case, the coating agent provides selectivity in recognition perhaps through processes involving differential opsonization, recognition and cell uptake. Clearly, the Kupffer cells of the liver are unable to recognise and remove the particles. A similar type of selectivity has been proposed for the same particles when coated with the related substance, Poloxamer 407 (Davis and Illum, 1987). Here, the intravenously injected particles are ignored by the liver but were subsequently retained in a non-cell-associated state in the bone marrow (Illum and Davis, unpublished observations).

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