International Journal of Pharmaceutics, 29 (1986) 53–65
Elsevier

IJP 00964

The effect of hydrophilic coatings on the uptake of colloidal particles by the liver and by peritoneal macrophages

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(Received September 3rd, 1985)
(Accepted October 16th, 1985)

Key words: polystyrene microspheres – hydrophilic coating – colloidal particles – poloxamer 188 and 338 – secretory immunoglobulin A – egg lecithin – phagocytosis – gamma scintigraphy

Summary

By coating polystyrene microspheres (60 nm in diameter) with hydrophilic materials such as secretory immunoglobulin A (SIgA), egg lecithin and non-ionic surfactants (poloxamer 188 and 338) it was possible to divert the microspheres away from the liver after intravenous injection into rabbits, as measured by gamma scintigraphy. The most profound effect was found using poloxamer 338 as the coating agent with a 48% reduction in the liver uptake for the coated microspheres and a concurrent significant uptake by the bone marrow not found for the uncoated microspheres. The effect of the coating materials on phagocytosis was also studied in vitro using a mouse peritoneal macrophage system. A rank order correlation for phagocytosis was found between the in vitro and in vivo data. In the presence of serum poloxamer 338, but not poloxamer 188 maintained the ability to suppress phagocytosis. The powerful dysopsonic and anti-adhesive properties of poloxamer 338 were attributed to the large anchoring hydrophobic group preventing displacement of the polymer from the particle surface by plasma proteins and the two identical hydrophilic groups preventing cell adhesion and subsequent phagocytosis (steric stabilization).

Introduction

When colloidal carrier particles are injected intravenously, they are normally cleared rapidly from the bloodstream and accumulate largely in the liver and spleen, within the cells of the reticuloendothelial system (Illum et al., 1982; Poste and Kirsh, 1983). Consequently, attention has been given to the use of carrier particles for the passive delivery of drugs to the reticuloendothelial system itself for the treatment of various parasitic, fungal and bacterial diseases such as leishmaniasis and leprosy (Alving, 1982). However, in order to reach organs or tissue sites other than the liver and spleen, it will be necessary to achieve a different pattern of distribution for the carrier particles in the organism. This can be achieved easily by blocking the reticuloendothelial system, for example by preinjection of placebo colloidal particles or with a reticuloendothelial depressant such as dextran sulphate 500. Thus, Illum et al. (1985) have studied the effect of a preinjection with polystyrene latex particles and dextran sulphate 500 on the distribution of a model carrier particle system in the rabbit using gamma scintigraphy. For both systems the effects of blocking resulted in a de-

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crease in liver and spleen uptake of the carrier particles from 90% to 30% and an increase in lung uptake from 5% to 60%. Similarly Proffitt et al. (1983) have reported recently that a 50% greater quantity of radioactive liposomes were deposited in tumours of mice, than for untreated controls when the reticuloendothelial system was blocked with unlabelled liposomes. However, this approach for the diversion of particles away from the liver and spleen, would not normally be applicable in clinical practice. Nevertheless it does provide basic information about the reticuloendothelial system in the animal model and the extent to which redistribution via blockade can occur.

The uptake of carrier particles by the phagocytic cells of the liver, spleen and bone marrow is determined mainly by the physicochemical characteristics of the particles; especially, their size, surface charge and surface affinity (hydrophobicity/hydrophilicity) (Illum and Davis, 1982; Van Oss et al., 1975). Hydrophobic particles will be removed from the circulation rapidly, while more hydrophilic particles would be expected to remain in circulation for longer periods of time (Van Oss et al., 1975). Thus it can be predicted that the coating of carrier particles with high molecular weight materials, that would give rise to a hydrophilic surface with a low surface charge and the opportunity for steric stabilization (Napper and Netschey, 1971), should give rise to a minimum uptake by the reticuloendothelial system, thereby allowing particles to reach other sites. Indeed coating of particles with negative or positive macromolecules can alter organ uptake considerably, as reported by Wilkins and Myers (1966), who found positively charged colloids to accumulate initially in the lungs and later the spleen, while negatively charged colloids were found in the liver and spleen. Jeppsson and Rossner (1975) and Geyer (1967) have studied emulsion systems and demonstrated that non-ionic surfactants of the Pluronic (poloxamer) series can modify the kinetics of blood clearance. Illum and Davis (1983, 1984) showed that blood clearance and organ distribution of polystyrene microspheres (1.24 μm and 60 nm in diameter) could be altered using Pluronic F108 (poloxamer 338) as the coating agent. The most dramatic effect on liver uptake was achieved for the smaller size particles.

In the present work we have investigated a wider range of coating materials, e.g. mixed phospholipids (lecithin), secretory immunoglobulin A (SIgA) and non-ionic surfactants all of which should give rise to a hydrophilic particle surface. Polystyrene microspheres, of sizes 160 and 60 nm in diameter, have been used as model carrier particles. Studies have been conducted in animal models as well as in isolated murine macrophages. The mouse peritoneal model has been chosen since others (Roerdink et al., 1984; Davis and Hansrani, 1985) have shown it to be useful for distinguishing between particles with different surface characteristics.

Experimental

Materials

Polystyrene microspheres (2.5% suspension, 60 nm, 160 nm and 5.25 μm mean diameter) used as model carrier particles were purchased from Polyscience, Northampton, U.K. The particle sizes were confirmed using either photon correlation spectroscopy (Malvern Instruments, Malvern, U.K.) or Coulter Counter (model TA II, Coulter Electronics, Dunstable, U.K.)

The polystyrene microspheres were surface-labelled with iodine-131 using a previously described irradiation technique (Illum et al., 1982). Any free [^{131}I]sodium iodide and water-soluble irradiation products were removed by dialysis. The labelling efficiency was found to be in the order of 30%.

Poloxamer 338 and 188 were obtained from Ugine Kuhlman, Bolton, U.K. and made up to 2% w/v aqueous solutions.

Egg lecithin (73% phosphatidylcholine, 15% phosphatidylethanolamine, 6% lysophosphatidylcholine, 2.1% lysophosphatidylethanolamine) was purchased from BDH, Poole, U.K. and dissolved in ethanol to give a concentration of 3% w/v.

Secretory immunoglobulin A (SIgA) from human colostrum was purchased from Sigma Chemicals, Dorset, U.K. and made up to 1% w/v aqueous solution.

Coating and characterization of particles

Polystyrene microspheres (60 nm and 5.25 μm) were coated by incubation with equal volumes of egg lecithin, SIgA and poloxamer solutions for 24 h. The concentrations of the coating agents were chosen such that at equilibrium the quantity of adsorbed material was in the plateau region of the respective adsorption isotherms (Tamamushi, 1983; Norde, 1979; Kayes and Rawlins, 1979). The 160 nm particles were studied only uncoated. The thicknesses of adsorbed layers of the various coating materials were determined using the 60 nm microspheres by means of photon correlation spectroscopy. Microspheres were highly diluted in double-distilled filtered water before measurement.

The surface charge on the coated and uncoated microspheres (60 nm in size) was determined by the method of particle microelectrophoresis using the technique of laser doppler anemmometry (Zeta Sizer, Malvern Instruments, Malvern, U.K.) as previously described (Douglas et al., 1985). Microspheres were diluted in 1 mM KCl solution and a total of 1000 measurements were performed on each sample. Zeta potentials were calculated from the mean mobility results (Henry equation, $K_a = 3$).

Animal experiments

Female New Zealand White rabbits (weight range 3–5 kg) were divided into groups of 3. The radiolabelled polystyrene microspheres were incubated with equal volumes of either egg lecithin, SIgA or poloxamer solutions for 24 h prior to injection. Each of three rabbits was injected intravenously via the marginal ear vein with 0.6 ml ($= 4 \times 10^{13}$ microspheres, 9.25 MBq) of polystyrene microsphere suspension coated with SIgA, egg lecithin, poloxamer 338 or poloxamer 188 or 0.3 ml ($= 4 \times 10^{13}$ microspheres, 9.25 MBq) of non-coated polystyrene microsphere suspension.

A control experiment ($n = 2$) was conducted in which poloxamer 338 solution 1% (0.3 ml) was administered via the marginal ear vein and immediately afterwards the uncoated microsphere suspension (0.3 ml) was administered via the contralateral ear vein.

The distribution of the labelled microspheres was followed using gamma scintigraphy (Maxi Camera II, Gamma Camera, International General Electric Company of New York). Dynamic studies (20 s duration) and static images (60 s duration), showing the distribution of the microspheres, were recorded for 15 min and then at suitable intervals over 8 days, respectively. The data were recorded and processed using a dedicated computer system as previously described (Illum et al., 1982). Regions of interest were created around the areas of the liver/spleen, the lung/heart and the left hind leg. Blood level activity was estimated during the time of the studies using a non-invasive gamma probe placed over the front paw of the animal (Illum et al., 1982).

Eight days after administration the rabbits were killed and the organs removed. The total activity in the different organ sites and in the carcass was determined using a large sample volume, well-type gamma-counting system (EG and G Ortec).

Mouse peritoneal macrophages

The test particles were polystyrene microspheres, 5.25 μm in diameter, which were dialyzed against distilled water for 3 days. These microspheres were manufactured in the same way as the much smaller microspheres used in the in vivo experiments (personal communication, Polyscience). Female MF1 mice weighing 20–25 g, were used to provide the peritoneal macrophages. The animals were killed by CO_2 asphyxiation, the peritoneal wall exposed and 5 ml of lavage medium (10 ml tissue culture Medium E199 concentrate (10 ×) (Flow Laboratories), 10 ml swine serum, 2.5 ml sodium bicarbonate 7.5%. 0.1 ml crystamycin, 6 mg heparin, 77.4 ml sterile water) injected into the peritoneal cavity followed by a smaller volume of sterile air. The peritoneal wall was gently massaged and the medium containing the macrophages was withdrawn and collected in a sterile container kept on ice. The exudates from several animals were routinely collected in this way and pooled. A cell count was conducted using a Coulter Counter (model TAII). The viability of the macrophages was tested by exclusion of tryptan blue and found to be in the order of 95%. The macrophage suspension was adjusted to a final cell count of 1.0×10^6 cells/ml and 1.25 ml of this suspension pipetted into each 30 mm dish to give

1.25×10^6 cells per plate. The plates were incubated at 37°C in 95% air/5% CO_2 for 3 h to permit macrophage adherence to the bottom of the plate. After adherence the medium was removed from the plates, the cells washed once with sterile PBS, 1.25 ml of cell culture medium added (10 ml Medium E199 concentrate (10 ×), 10 ml swine serum, 2.5 ml sodium bicarbonate, 0.1 ml crystamycin, 10 mg L-glutamine and 79.9 ml sterile water), and the plates incubated at 37°C in 95% air/5% CO_2 for 24 h. After incubation the medium was removed and the cells washed once with sterile PBS. Then 2.5 ml cell culture medium (without swine serum) containing the appropriate number of coated or uncoated microspheres (5 particles per macrophage) determined by a particle-to-cell ratio experiment (see below) was added to each plate and the plates incubated in groups of 3 for 10, 20, 30, 45 and 60 min, as determined beforehand by the time course experiment (see below). In order to investigate the role of opsonic factors, studies were undertaken with added serum for uncoated microspheres and for microspheres coated with poloxamers 338 and 188. Before counting the number of particles phagocytosed by the macrophages, the media was removed from the plates, the cells washed 2 times with sterile PBS and fixed with methanol for 5 min. Then the cells were stained with Geimsa (1 : 10) for 15 min and washed with water. The plates were left to dry and the number of microspheres phagocytosed by the macrophages was counted for a total of 100 macrophages using a light microscope at a magnification of 500 times. The experiments were done in triplicate and results were expressed as the number of microspheres phagocytosed by a 100 macrophages.

Determination of particle-to-cell ratio

Cell cultures containing 1.25×10^6 cells were incubated with 1.25 ml of cell culture medium (without swine serum) containing different concentrations of uncoated microspheres, e.g. 5, 10, 25 and 50 particles per cell equivalent to 6.25×10^6, 12.5×10^6, 31.25×10^6 and 62.5×10^6 particles, respectively. After incubation for 2 h at 37°C in 95% air/5% CO_2 the number of particles phagocytosed in each of a 100 macrophages was determined. The experiments were performed in triplicate. The optimum particle-to-cell ratio for counting was determined from the phagocytosis versus particle concentration profile and was found to be 5 particles per cell.

Determination of time course of phagocytosis

Using the appropriate microsphere-to-cell ratio (5 particles per cell) as determined above, the cells were incubated with the microspheres for different time periods, to determine the incubation time for optimal particle uptake for counting. The time periods chosen were 10, 30, 60 and 120 min. All experiments were performed in triplicate. For these experiments the optimal time for incubation was found to be 60 min.

Results

Characterization of uncoated and coated particles

Particle size analysis and microelectrophoresis experiments conducted on the coated and the uncoated microspheres (60 nm) demonstrated that the microspheres obtained a coating of the relevant material after incubation. In each case particle size and/or microelectrophoretic data (zeta potential) could be compared with predicted values obtained from literature data on the thickness of adsorbed films or by comparison with other colloidal particles (fat emulsion) carrying the same coating material (Table 1). All coatings gave an increase in the hydrodynamic diameter of the polystyrene microspheres (for the lecithin system particle size analysis was not possible due to the presence of suspended lecithin particles). Adsorbed SIgA produced a small decrease in the electrophoretic mobility (zeta potential), while much larger decreases were seen for the two poloxamer systems. Adsorbed egg lecithin gave a rise in mobility. The derived value for zeta potential of −30.0 mV corresponds closely with that obtained for emulsion droplets (Intralipid −33.3 mV) stabilized with egg lecithin.

Distribution of microspheres in vivo

Evaluation of the scintigraphic data, obtained from the gamma camera, showed that the coating

TABLE 1

ELECTROPHORETIC MOBILITIES (EPM) AND ZETA POTENTIALS (ZP) FOR POLYSTYRENE MICROSPHERES UNCOATED AND COATED WITH DIFFERENT COATING MATERIALS

Coating material	Particle diameter (nm)		EPM ($\mu m \cdot cm \cdot s^{-1} \cdot V^{-1}$)	ZP (mV)
	Measured ± S.E.M.	Predicted		
None	63.1 ± 0.34	60	−2.93	−24.18
SIgA	108	123	−2.70	−22.27
Lecithin	–	–	−3.63	−29.98
Poloxamer 188	74.7 ± 1.04	78	−1.51	−12.47
Poloxamer 338	95 ±0.28	90	−1.74	−14.34
Fat emulsion	–	–	−3.57	−33.26

of polystyrene microspheres with SIgA and lecithin altered the organ distribution of the microspheres only to a small extent (Fig. 1). Uncoated microspheres were taken out of the circulation rapidly and were deposited mainly in the liver and spleen with approximately 90% in these two organs and 5% in the lung/heart region. Coating the microspheres with SIgA and lecithin resulted in minor decreases in liver/spleen uptake (i.e. 2% and 14%, respectively) and minor increases in the lung/heart region.

The effect of coating the polystyrene microspheres with the non-ionic surfactants poloxamer 188 and 338 on their distribution in the body is also shown in Fig. 1. For the microspheres coated with poloxamer 188 the decrease in liver/spleen uptake was of the order of 20%. While coating the microspheres with poloxamer 338 had a more profound effect on the distribution. Although some microspheres were still found in the liver and spleen, the total uptake in these two organs was reduced to 46% with a much larger activity being found in the lung/heart region (30%). Control experiments showed that poloxamer 338 given immediately before the uncoated microspheres via the contralateral ear had no effect on the distribution. Scintiscans demonstrated that the poloxamer 338-coated microspheres had been distributed to the bone marrow. The rate and extent of uptake into the bone marrow of the left hind leg were determined by creating a region of interest around the image of the leg (Fig. 2). The rate of uptake could be characterized by a half-life of about 0.5 min. In contrast, the microspheres coated with poloxamer 188 did not provide a discernable image of the leg and creation of an appropriate region of interest showed that the uptake of microspheres into the bone marrow in the hind leg was not significant. Activity localized within the bone marrow will also appear in those regions of interest that are high in bony tissue. Thus, the region of interest created around the heart/lung will also include activity associated with the ribs and the spinal column. Scintigraphic studies in rabbits, using the bone scanning agent ^{99m}Tc-labelled poly-

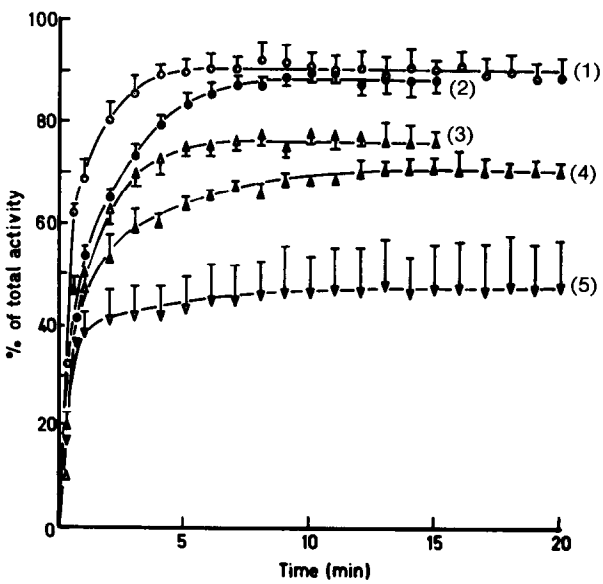

Fig. 1. Uptake of labelled polystyrene microspheres (60 nm) by the rabbit liver (n = 3). Key: (1), uncoated microspheres (MS); (2), MS coated with SIgA; (3), MS coated with lecithin; (4), MS coated with poloxamer 188; (5), MS coated with poloxamer 338.

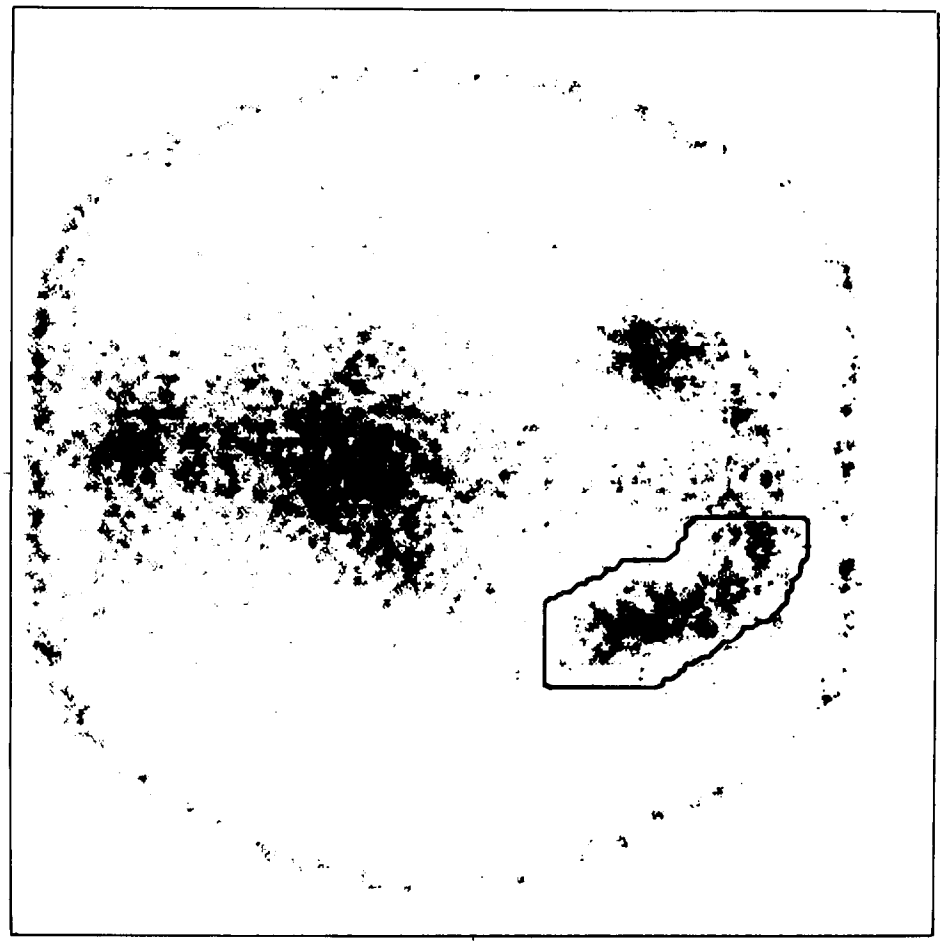

Fig. 2. Scintiscan showing image of rabbit following administration of labelled polystyrene microspheres (60 nm) coated with poloxamer 338 and region of interest around hind leg.

phosphate, showed that the relative uptakes to bone regions for the hind leg and lung/heart region were in the ratio 1 : 1.2. Therefore, assuming that the relative distribution within the bone marrow for coated microspheres occurs in a similar manner to that for labelled polyphosphate, an estimation can be made for uptake of microspheres coated with poloxamer 338 in the lung and heart alone. This was of the order of less than 1% of administered dose.

The kinetics of uptake of labelled microspheres into liver and spleen were calculated using data in Fig. 1, by fitting them to a first-order equation. As can be seen from Table 2 none of the coating agents had a significant effect on the rate of uptake except SIgA. Even with this material the time for 50% uptake was as short as 1 min. No significant difference could be found between the rate of uptake of uncoated microspheres of diameters 60 nm and 160 nm.

The deposition of the coated and the uncoated microspheres in the various organs 8 days after administration is shown in Table 3. For the microspheres coated with SIgA, lecithin and poloxamer 188 no significant differences were found between the organ distribution of coated and uncoated microspheres except for higher activity levels in the lungs for lecithin and poloxamer 188 coated

TABLE 2

THE RATE OF UPTAKE IN THE LIVER OF UNCOATED AND COATED POLYSTYRENE MICROSPHERES AFTER INTRAVENOUS ADMINISTRATION

Polystyrene microspheres	Uncoated		Coated with:			
	60 nm	160 nm	SIgA	Lecithin	Poloxamer 188	Poloxamer 338
Rate of uptake in the liver (T_{50}%) (s)	50.3(±1.5)	41.0(±8.0)	66.1(±2.9)	50.3(±0.4)	65.8(±9.9)	55.0(±17.0)

The data are expressed as the times for 50% uptake ($T_{50\%}$) (±S.E.M.).

microspheres. However, the microspheres coated with poloxamer 338 retained the distribution pattern demonstrated by the gamma camera study with higher levels of activity in the lungs, spleen and carcass and a corresponding reduction in the quantity of activity reaching the liver. The low level found in the lungs confirms the finding above that the greater proportion of the activity recorded in the lung/heart region in the gamma camera study was due to microspheres deposited in the bone marrow of the ribs and spinal column.

The estimated blood clearance profiles expressed in terms of paw activity for the uncoated and coated systems consisted of two phases; a rapid initial decrease followed by a second very much slower phase. Data for the paw level activity after 1 min and the clearance ($T_{50\%}$) of those microspheres remaining in the paw after 1 min are presented in Table 4. For poloxamer 188 and 338 the $T_{50\%}$ values for the microspheres remaining in the paw were 1.7 days and 5 days, respectively, compared to 1 h for the uncoated microspheres and microspheres coated with SIgA and lecithin. Furthermore, the amount of microspheres remaining in the paw after 1 min was significantly higher for the two particle systems coated with poloxamer.

Interaction of microspheres with macrophages in vitro

The uptake of the coated and uncoated microspheres by mouse peritoneal macrophages is shown in Fig. 3. The highest rate of uptake of microspheres by the macrophages was found for the uncoated microspheres while the microspheres coated by the poloxamer systems gave the lowest rate of uptake. A rank order correlation between the in vitro and in vivo studies was demonstrated by expressing the data in terms of the relative uptake where the uncoated microspheres are taken as a 100% (Fig. 4).

The effect of added serum was examined for the

TABLE 3

DEPOSITION OF UNCOATED AND COATED POLYSTYRENE MICROSPHERES IN THE VARIOUS ORGANS 8 DAYS AFTER INTRAVENOUS ADMINISTRATION IN RABBITS

	Lung	Heart	Kidney	Spleen	Liver	Carcass
Polystyrene microspheres	0.15(±0.01)	0.11(±0.01)	0.22(±0.02)	1.45(±0.2)	59.5(±6.9)	38.6(±7.1)
Polystyrene microspheres coated with SIgA	0.20(±0.05)	0.13(±0.01)	0.26(±0.01)	4.4 (±0.8)	61.7(±7.7)	33.3(±6.9)
Polystyrene microspheres coated with lecithin	2.3 (±0.6)	0.26(±0.02)	0.37(±0.07)	3.6 (±0.5)	55.7(±3.7)	37.8(±3.6)
Polystyrene microspheres coated with poloxamer 188	1.6 (±0.1)	0.26(±0.01)	0.34(±0.02)	2.3 (±0.1)	60.9(±2.4)	34.7(±2.6)
Polystyrene microspheres coated with poloxamer 338	0.51(±0.03)	0.22(±0.01)	0.34(±0.03)	0.93(±0.19)	30.2(±5.5)	67.9(±5.7)

The data are expressed as the percentage of total activity (±S.E.M.)..

TABLE 4

BLOOD LEVEL ACTIVITY EXPRESSED AS PAW ACTIVITY 1 min AFTER ADMINISTRATION OF UNCOATED AND COATED POLYSTYRENE MICROSPHERES AND THE CLEARANCE (T_{50}%) OF THE MICROSPHERES REMAINING AFTER 1 min

System	Paw level activity after 1 min, (percentage of initial activity)	$T_{50\%}$ for paw level activity remaining after 1 min
Polystyrene microspheres (60 nm)	4.6%	1 h
Polystyrene microspheres coated with SIgA	2.6%	1 h
Polystyrene microspheres coated with lecithin	4.1%	1 h
Polystyrene microspheres coated with poloxamer 188	11.6% *	1.7 days *
Polystyrene microspheres coated with poloxamer 338	8.8% **	5 days **

* Significant difference from uncoated polystyrene microspheres ($0.05 > P > 0.02$).
** Significant difference from uncoated polystyrene microspheres ($0.02 > P > 0.01$).

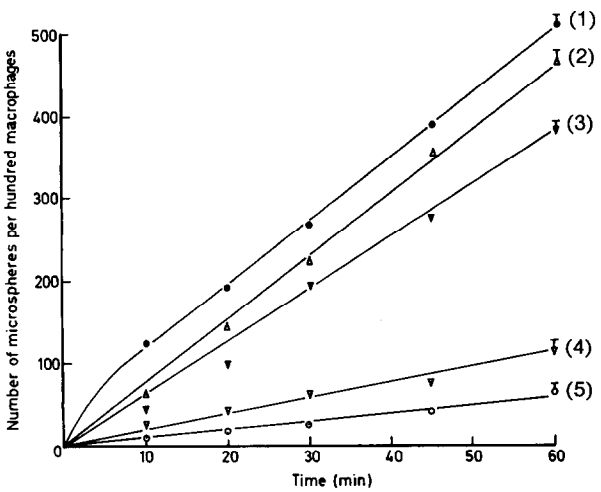

Fig. 3. Uptake of polystyrene microspheres (5.25 μm) by mouse peritoneal macrophages. Typical error bars are shown. Key: (1), uncoated microspheres (MS); (2), MS coated with SIgA; (3), MS coated with lecithin; (4), MS coated with poloxamer 188; (5), MS coated with poloxamer 338.

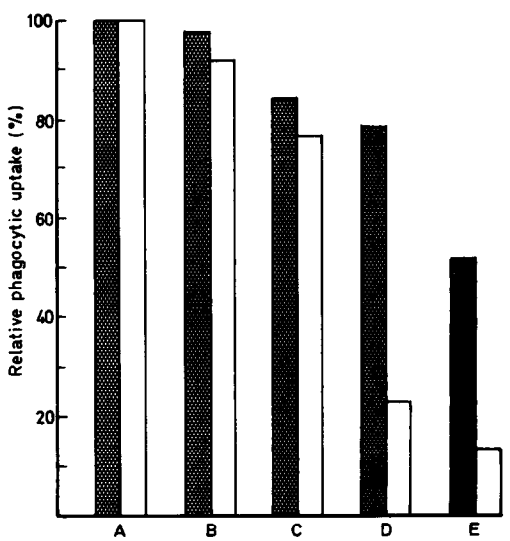

Fig. 4. In vitro–in vivo correlation of phagocytic uptake of polystyrene microspheres by macrophages. Key: filled boxes, in vivo data (rabbit liver); unfilled boxes, in vitro data (mouse peritoneal macrophages). A = uncoated microspheres (MS); B = MS coated with SIgA; C = MS coated with lecithin; D = MS coated with poloxamer 188; E = MS coated with poloxamer 338.

TABLE 5

THE EFFECT OF ADDED SERUM ON THE UPTAKE OF UNCOATED AND COATED POLYSTYRENE MICROSPHERES

Polystyrene microspheres	Uncoated	Coated with:	
		Poloxamer 188	Poloxamer 338
Serum not present	100%	22%	14%
Serum present	100%	90%	23%

Data expressed as the percentage of uptake of uncoated microspheres.

uncoated microspheres and the microspheres coated with poloxamer 338 and 188 (Table 5). The serum had no significant effect on the uptake of the uncoated microspheres and those coated by poloxamer 338. However, the observed marked reduction in phagocytosis caused by coating with poloxamer 188 in the absence of serum, was no longer apparent when serum was present.

Discussion

Coating of microspheres

The results of the particle size analysis and the electrophoretic mobility studies demonstrated clearly that the various coating agents were taken up onto the surface of the polystyrene microspheres and were not desorbed in vitro in water or electrolyte solutions (Table 1). Furthermore, previous studies (Illum and Davis, 1983) have indicated that when uncoated systems and systems coated with poloxamer 338 are incubated with plasma the zeta potentials are reduced to about −10 mV for the uncoated and 0 mV for the coated microspheres, respectively. The measured thicknesses of adsorbed layers of poloxamer and SIgA agree well with previous experimental determinations and predictions based upon similar studies conducted on related immunoglobulins (Kayes and Rawlins, 1979; Bagchi and Birnbaum, 1981). As expected, the poloxamers and the SIgA reduced the electrophoretic mobility (zeta potential) (Kayes and Rawlins, 1979; Edebo et al., 1980). This reduction can be associated with the hydrophilic and nonionic character of these materials when adsorbed to surfaces (polyoxyethylene groups for the poloxamers and carbohydrate groups for the SIgA). Polystyrene microspheres coated with adsorbed egg lecithin were similar in their electrophoretic behaviour to their emulsion counterpart. The increase in zeta potential can be associated with ionizable phospholipids present as minor components in the egg lecithin (Davis, 1983).

In vivo experiments

The various selected coating agents demonstrated different abilities to divert particles away from the liver (Fig. 1). Poloxamer 338 provided the most dramatic effect, not only in reducing liver uptake but also in giving localization of microspheres in the bone marrow. Initially, poloxamer 188 gave a significant reduction in liver uptake but this did not persist at longer time periods (8 days) when organ levels were analyzed, and in this respect was similar to SIgA and lecithin. Interestingly, for all systems including poloxamer 338, the rate of uptake of microspheres was little or not affected by the coating materials. These results confirm our earlier studies on coating effects (Illum and Davis, 1984).

Foreign particles such as polystyrene microspheres are normally taken up by the reticuloendothelial cells of the liver and spleen after intravenous injection. This rapid and efficient removal is a result of two interrelated processes; the first is the coating of the particles by blood components (opsonization) that renders them recognizable by phagocytic cells. These opsonic materials include immunoglobulin G and fibronectin. The second is the adhesion of the particles to the surface of the phagocytic cells and their subsequent engulfment. It has long been known that the physical properties of the particles can affect both processes. However, it is not normally possible to resolve opsonic and adhesional contributions from data obtained by in vitro experiments. A number of authors have claimed that surface hydrophobicity is a key factor (Van Oss et al., 1975). Hydrophobic particles are phagocytosed more rapidly than hydrophilic particles. It has been suggested that opsonization leads to the creation of a more hydrophobic surface and that very hydrophobic particles do not need to be opsonized before phagocytosis. While this physicochemical approach is useful in explaining non-specific mechanisms of particle recognition and uptake it must be borne in mind that more specific receptor mediated processes involving immunoglobulins, complement, etc., can occur. If a particle is made hydrophilic it is expected that it will reduce opsonization and may prevent adhesion and phagocytosis. For example, Edebo et al. (1980) have suggested that SIgA will have a dysopsonization effect due to the fact that this secretory component has a high content of carbohydrate, up to 15.6% (Kobayashi, 1971) and that this material is exposed on the

SIgA molecule (Brandtzaeg, 1974). Thus, binding of SIgA to *Salmonella typhimurium* reduced significantly the phagocytosis in vitro of this bacterium by polymorphonuclear leucocytes (Magnusson et al., 1979). The slight reduction in liver uptake obtained in the present study may be a reflection of this effect. Similarly, Roerdink et al. (1983) have examined whether surface charge can play a role in the phagocytosis of opsonized liposomes. The presence of negatively charged lipids within the liposome profoundly suppressed the uptake by mouse peritoneal macrophages. This charge effect could be an explanation of the reduction in uptake in the liver observed when particles were coated with egg lecithin that contains significant amounts of ionic phospholipids. For both the SIgA- and lecithin-coated systems the suppression of liver uptake was not particularly impressive even so far as initial suppression was concerned and was lost by 8 days. We believe that a displacement of the adsorbed coating material is likely to occur in vivo by plasma components that have a stronger affinity for the particle surface.

Non-ionic surfactants, such as the block copolymers of the poloxamer series, are known to adsorb strongly to hydrophobic surfaces and are not displaced by dilution in aqueous buffer. For this reason, and the fact that they have low toxicity, they were selected for study in the present work. Not only should they provide a hydrophilic surface and a dysopsonic effect but should also minimize particle adhesion through a process of steric stabilization. A schematic diagram of the adsorption of poloxamer is shown in Fig. 5. The hydrophobic block made up of polyoxypropylene is bound to the hydrophobic surface while the two hydrophilic blocks consisting of polyoxyethylene are largely sticking out in the external environment. It can be reasoned from molecular weight and hydrophilic–hydrophobic balance that poloxamer 338 should be firmly anchored to the particle surface, i.e. less liable to displacement by blood components and provide a larger steric barrier (excluded volume effect; Silberberg, 1984). Indeed we found that both poloxamer materials gave rise to a substantial reduction in liver uptake and a delayed clearance of microspheres from the blood. Poloxamer 338 was much more effective in

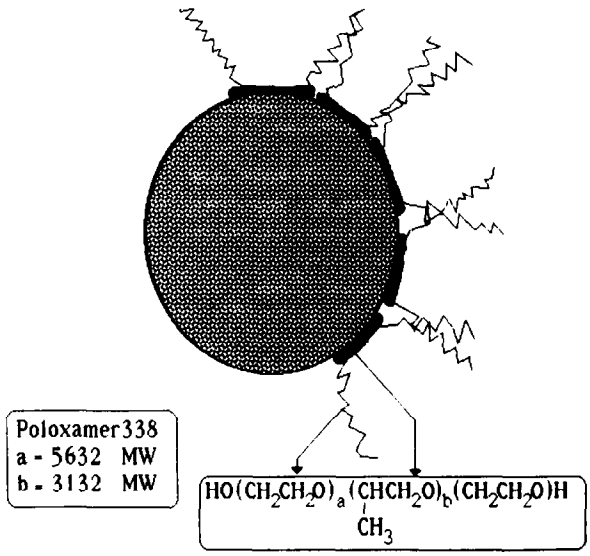

Fig. 5. Schematic diagram showing the coating of a polystyrene microsphere by poloxamer.

this respect than poloxamer 188 in that coated microspheres reached the bone marrow in significant quantities and that this altered distribution was still evident 8 days after administration. Leu et al. (1984) have recently reported similar studies using poloxamer 188 as the coating agent on polymethylmethacrylate nanoparticles and rats as the animal model. They found a similar but greater extent of diversion away from the liver to other sites. The reason for the dissimilarity between the extents of diversion for the work of Leu et al. (1984) and the present study can probably be found in differences in the injected dose and the nature of the two animal models (rat and rabbit, respectively). The smaller blood volume in the rat and the larger quantity of poloxamer 188-coated microspheres may result in less possibility in displacement of the poloxamer coating.

The suppression of the liver uptake using microspheres coated by poloxamer 338 is similar to that seen for the experiments where the Kupffer cells are preblocked (Illum et al., 1985). It is believed that the residual activity found for both the poloxamer 338 and the blocking experiments may reflect the complete suppression of uptake of microspheres by the Kupffer cells and the ex-

istence of concurrent endocytic processes by endothelial cells and hepatocytes. Such an alternative parallel mechanism would explain an unaltered rate of uptake for the smaller amounts of microspheres sequestered for coated and blocked systems. Praaning-Van Dalen et al. (1982) have shown that hepatocytes and endothelial cells can play a significant role in the endocytosis of foreign particles when the particles are below 0.2 μm. Thus, it would appear that the coating agent poloxamer 338 may have suppressed the phagocytic process and not other endocytic mechanisms. For example, hepatocytes and endothelial cells can take up particles by bristle-coated micropinocytosis.

The coated particles not taken up by the liver can find their way to the bone marrow. Pinocytic uptake mechanisms are also thought to operate in this case. More specifically, Yoshida et al. (1984) have reported that the sinusoidal endothelial cells of the rabbit liver took up colloidal carbon through the formation of bristle-coated vesicles but failed to ingest polystyrene particles of 0.11 μm or larger. On the other hand, the sinusoidal endothelial cells of the rabbit bone marrow took up carbon and polystyrene particles varying from 0.11 to 2.02 μm in diameter through the formation of two kinds of vesicles at the luminal surface: bristle-coated vesicles and "multiparticle-pinocytotic vesicles".

The redirection of microspheres to the bone marrow could have application in drug targeting, i.e. B-cells lymphoma.

In vitro experiments

The uptake of polystyrene microspheres by mouse peritoneal macrophages is dependent on whether the microspheres are uncoated or coated with hydrophilic materials. The presence or absence of serum is also an important factor. Dealing first with the data obtained in the absence of serum coating the microspheres with SIgA and lecithin affected the rate of phagocytosis to a small extent. In contrast, when the microspheres were coated with poloxamer the uptake was greatly diminished. A rank order correlation between the in vitro and in vivo data can be demonstrated by expressing the results in terms of the relative uptake, where the uncoated microspheres are taken as a 100% (Fig. 4).

The results for the poloxamer systems and for egg lecithin can be compared with those given recently by Davis and Hansrani (1985). Emulsions stabilized by egg lecithin were phagocytosed quite rapidly by mouse peritoneal macrophages and this was dependent upon the nature of minor components within the lecithin. However, for emulsions stabilized with the two poloxamers almost no uptake was observed. Such results support the suggestion that hydrophilic coatings on colloidal particles can affect cell–particle adhesion through an ionic repulsion effect and steric stabilization. Apparently, the latter process will have the more profound effect.

In the presence of serum, poloxamer 338 maintained its ability to suppress phagocytosis (Table 5). However, for the lower molecular weight material poloxamer 188 this effect had largely disappeared. This is in correlation with the in vivo data showing a short-term but not a long-term (after 8 days) reduction in uptake by the liver/spleen of microspheres coated with poloxamer 188. These results support the hypothesis that agents employed for dysopsonic and anti-adhesive properties (steric stabilization) must satisfy two requirements; a sufficiently large hydrophobic anchoring moiety to prevent displacement by plasma proteins and a sufficiently large hydrophilic moiety or moieties to prevent adhesion.

Conclusions

Coating small colloidal particles with hydrophilic materials such as lecithin, SIgA and nonionic surfactants can reduce the uptake of such particles by the liver when administered intravenously into rabbits. The dramatic and lasting effect found for the block copolymer poloxamer 338 can be attributed to the fact that it has a large hydrophobic group that anchors the polymer to the surface of the particles and large hydrophilic groups that minimize opsonization and particle–cell adhesion. With this coating material, particles not removed by the liver were taken up to a significant extent by the bone marrow.

Experiments conducted using mouse peritoneal macrophages confirmed that hydrophilic coatings

could lead to a reduction in the rate of phagocytosis. A rank order correlation was found between the in vivo and in vitro data.

Poloxamer 338 was not displaced from the particles in the presence of serum, suggesting that this coating agent has both dysopsonic and anti-adhesive (steric stabilization) properties.

Acknowledgements

This study was supported by Nato Science Foundation Double Jump Program.

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