International Journal of Pharmaceutics, 68 (1991) 121–126

ADONIS 0378517391000818

IJP 02292

The effect of poloxamer-407 on liposome stability and targeting to bone marrow: comparison with polystyrene microspheres

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(Received 3 August 1990)
(Accepted 20 August 1990)

Key words: Bone marrow; Liposome; Poloxamer; Reticuloendothelial system; Photon correlation spectroscopy

Summary

In accordance with our previous studies in the rabbit (Illum, L. and Davis, S.S., *Life Sci.*, 40 (1987) 1553–1560), coating of model polystyrene microspheres (70 nm in size) with poloxamer-407 was found to prevent their hepatic sequestration and to redirect a significant proportion of intravenously injected particles to the bone marrow in rats. In contrast, preincubation of a stable and 'solid' small unilamellar liposome preparation (DSPC/Chol/DCP in a molar ratio of 7 : 7 : 1) with poloxamer-407 provided no evidence of poloxamer penetration or adsorption onto the vesicles, as determined by photon correlation spectroscopy and laser doppler anemometry. Further, no significant difference on biodistribution of poloxamer-preincubated liposomes was observed in comparison to control vesicles following intravenous administration into rats.

Introduction

A major problem in targeting of intravenously injected colloidal drug carriers like liposomes and microspheres to the mononuclear phagocytes of the bone marrow is their rapid and efficient removal by the Kupffer cells of the liver and macrophages of the spleen (Bradfield, 1984). The removal of particulate matters from circulation by the host defence system depends on a number of parameters such as the particle size, surface characteristics and the presence of organ-specific opsonins (Saba, 1970; Gabizon and Papahadjopoulos, 1988; Moghimi and Patel, 1988, 1989). However, alteration in any of the above parameters can dramatically affect the biodistribution of the intravenously injected drug carriers among the organs of the reticuloendothelial system (RES). For example, previous studies from this laboratory demonstrated that the uptake of intravenously injected polystyrene microspheres and oil in water emulsions by Kupffer cells can be prevented by means of a hydrophilic polymeric coating (Illum et al., 1987, 1989). In this respect, using coating materials such as poloxamine 908, it was possible to keep polystyrene particles in circulation for extended periods of time in the rabbit (Illum et al., 1987). On the other hand, intravenous administration of polystyrene particles, smaller than 100 nm in diameter, coated with their poloxamer 338 or 407 resulted in their deposition in the bone

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marrow (Illum and Davis, 1984, 1987). Among these poloxamers, poloxamer 407 was more effective in diverting the microspheres to the bone marrow of the rabbit (Illum and Davis, 1987). This option may provide opportunities for the intravascular delivery of therapeutic agents for the treatment of diseases and disorders of the bone marrow as well as means for protection, stimulation or suppression of the marrow stem cell activity, if biodegradable drug carriers such as liposomes can be coated with poloxamer 407 (Moghimi et al., 1990).

Recently, Jamshaid et al. (1988) demonstrated that coating of small unilamellar liposomes (SUV) of egg phosphatidylcholine with polyoxyethylene-polyoxypropylene block copolymers (poloxamers) is possible. However, coating of such vesicles with poloxamers resulted in rapid leakage of their entrapped aqueous materials (Jamshaid et al., 1988). Hence, the present investigation was conducted to examine the possibility of coating of stable 'solid' SUVs with poloxamer 407 and to ascertain whether such system would demonstrate the same characteristics as found for non-biodegradable polystyrene microspheres following intravenous administration into rats. Solid liposomes have long circulatory half-lives (Senior, 1987) and recently it has been suggested that these vesicles may attract a serum dysopsonin which hinders their effective uptake by Kupffer cells of the liver (Moghimi and Patel, 1989). Thus, the possibility of coating such vesicles with poloxamer 407 may provide a better approach for their site-specific targeting to the bone marrow.

Materials and Methods

Materials

Distearoylphosphatidylcholine (DSPC), cholesterol (Chol), and dicetyl phosphate (DCP) were purchased from Sigma (U.K.). Polystyrene microspheres were obtained from Polysciences (U.K.). Poloxamer 407 was obtained from BASF Corp. (U.S.A.). [^{14}C-*carboxylic acid*]Inulin and Na^{125}I were purchased from Amersham International (U.K.).

Liposome preparation

Negatively charged multilamellar liposomes containing ^{14}C-labelled inulin were prepared as described earlier (Moghimi and Patel, 1989) from a mixture of DSPC/ChoL/DCP in a molar ratio of 7 : 7 : 1. For preparation of SUVs, this liposome suspension was sonicated for 60 min using a Dawe sonicator (type 7532-1A) equipped with a microtip probe (20% output power) at 50 °C. After sonication, the liposome preparation was allowed to stand at 50 °C for at least 1 h. The resulting suspension was centrifuged for 10 min at 10 000 × *g* to remove probe-derived titanium particles and chromatographed on sepharose-4B column equilibrated with phosphate buffered saline (PBS), pH 7.4, to remove non-entrapped [^{14}C]inulin.

Labelling of polystyrene particles

The polystyrene microspheres were surface labelled with Na^{125}I as described earlier (Illum and Davis, 1984). Any free Na^{125}I and water-soluble irradiation products were removed by dialysis. The labelling efficiency was found to be in the order of 25–30%.

Coating and characterization of particles

Polystyrene microspheres (70 nm) and small unilamellar liposomes (90-100 nm) were incubated in 0.85% w/v poloxamer 407 at room temperature for 24 h. The thickness of the adsorbed layer of poloxamer 407 on microspheres and liposomes were determined by means of photon correlation spectroscopy (Malvern Instruments, type LGK-7626). The surface charge on the particles was measured by means of laser doppler anemometry using a Zeta Sizer (Malvern Instruments).

Assessment of liposome stability

For the study of the structural integrity of liposomes in the presence of poloxamer and serum, 0.5 ml of fresh rat serum was mixed with 0.3 mg of either poloxamer treated or untreated [^{14}C]inulin-entrapped SUVs in a total volume of 1.0 ml in 10 mM PBS, pH 7.4, and incubated for 30 min at 37 °C. In control experiments serum was replaced with the same volume of PBS. After incubation 0.5 ml of mixture was passed through a Sepharose 4B column equilibrated with PBS to

separate the free and liposome-associated [^{14}C]inulin.

Animal studies

Groups of three to four male Wistar rats, 160 ± 10 g, were injected intravenously with 0.2 ml (20 mg lipid) of either poloxamer treated or control small unilamellar liposomes via the tail vein. Blood samples of 20 μl were taken from the tail vein at various time intervals and the animals were killed at 60 min after liposome injection. The livers were excised after perfusion through the portal vein with 40 ml of PBS, and organs such as spleen, lung, kidneys, and femoral bones were also collected for isotope measurement.

After removal all tissues were digested with 33% KOH overnight, 100 μl of samples were neutralized with 1.3 M HCl and the radioactivity was measured by using Optiphase 'Hi-Safe 3' (LKB) scintillant in an LKB scintillation counter.

In a further series of experiments rats were injected intravenously via the tail vein with about 10^{13} polystyrene microspheres labelled with ^{125}I. 1 h after the injection rats were killed and the radioactivity associated with blood, liver, spleen, lung, kidneys, bladder, and femoral bones were determined in an LKB gamma counter. To determine the amount of liposomes or polystyrene microspheres in the blood, we assumed a total blood volume per rat of 7.5% body weight (Patel et al., 1983).

Results

The adsorption characteristics of poloxamer 407 on both polystyrene microspheres and liposomes are presented in Table 1. Preincubation of microspheres with poloxamer 407 resulted in an apparent layer thickness of about 8 nm on the surface of the microspheres and a marked decrease in their zeta potential. In contrast, no significant difference in size and zeta potential between poloxamer pretreated and control liposomes was detected.

TABLE 1

Effect of poloxamer 407 on the size and zeta potential of liposomes and polystyrene microspheres

Treatment	Size (nm) (±SE)	Zeta potential (mV)
(A) Liposomes		
Control	107.3 ± 2.8 [a]	−7.9
Poloxamer treated	106.4 ± 3.6 [a]	−5.3
(B) Polystyrene microspheres		
Control	76.5 ± 1.5 [b]	−72.8
Poloxamer treated	84.7 ± 1.3 [b]	−15.0

For experimental details see Materials and Methods.
[a] Polydispersity index < 0.1.
[b] Polydispersity index < 0.05.

The effect of poloxamer on the stability of small unilamellar liposomes was determined in terms of release of the entrapped aqueous marker [^{14}C]inulin during in vitro incubation both in the absence and presence of 50% fresh rat serum. The results in Table 2 shows that more than 80% of the entrapped marker remains associated with liposomes after pretreatment with poloxamer and in the presence of serum.

The results in Fig 1 demonstrate the biodistribution of poloxamer treated vesicles in comparison to uncoated liposomes following intravenous administration into rats. The results indicate that the pretreatment of liposomes with poloxamer 407 produces no significant difference from the usual clearance pattern of the uncoated liposomes. In contrast precoating of polystyrene microspheres with poloxamers prevented their hepatic sequestration and increased femoral and splenic uptake

TABLE 2

The effect of poloxamer-407 on leakage of liposome-entrapped [^{14}C]inulin in the absence and presence of rat serum

Liposomes	% of initial [^{14}C]inulin associated with liposomes	
	Buffer	Serum
Poloxamer untreated	86	87
Poloxamer treated	80	85

For experimental details see Materials and Methods.

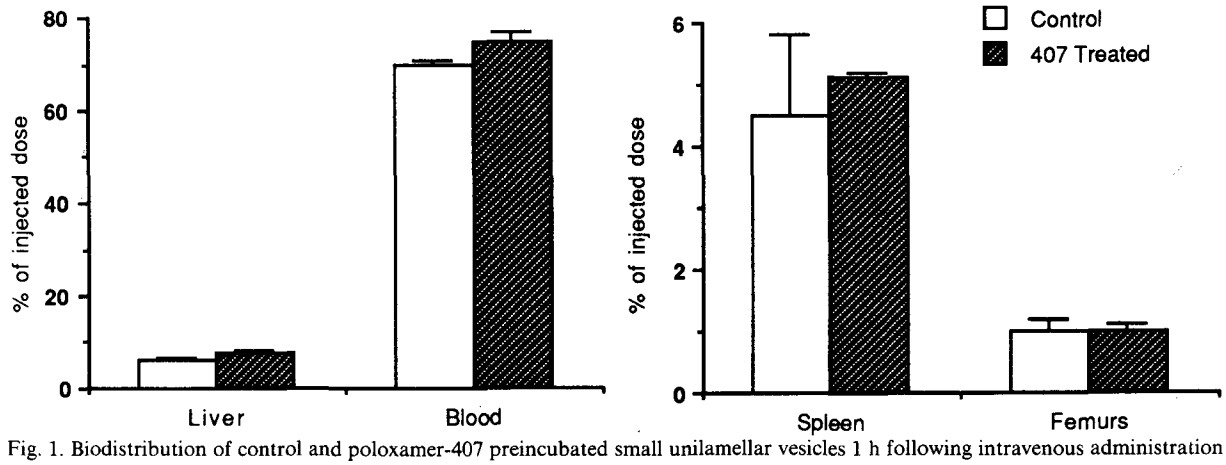

Fig. 1. Biodistribution of control and poloxamer-407 preincubated small unilamellar vesicles 1 h following intravenous administration into rats.

by 3- and 2-fold, respectively, as compared to uncoated particles (Fig 2).

Discussion

Recently, Jamshaid et al. (1988) suggested that polyxamers can penetrate the lipid bilayer of fluid egg PC liposomes and project their polyoxyethylene groups from the vesicles surface. The apparent adsorbed layer thickness on the surface of small unilamellar egg PC vesicles was in the order of 10 nm after 24 h incubation at room temperature in a concentration range of 0.01–0.1% (w/v) of poloxamer 338 (Jamshaid et al., 1988). In contrast, using a 'solid' phospholipid with a high phase transition temperature, DSPC ($T_c = 55\,^\circ$C), we have been unable to detect any significant change in size and electrophoretic mobility among poloxamer treated and control vesicles. Even incorporation of approx. 50 mol% cholesterol into the liposomal bilayer, which leads to fluidising

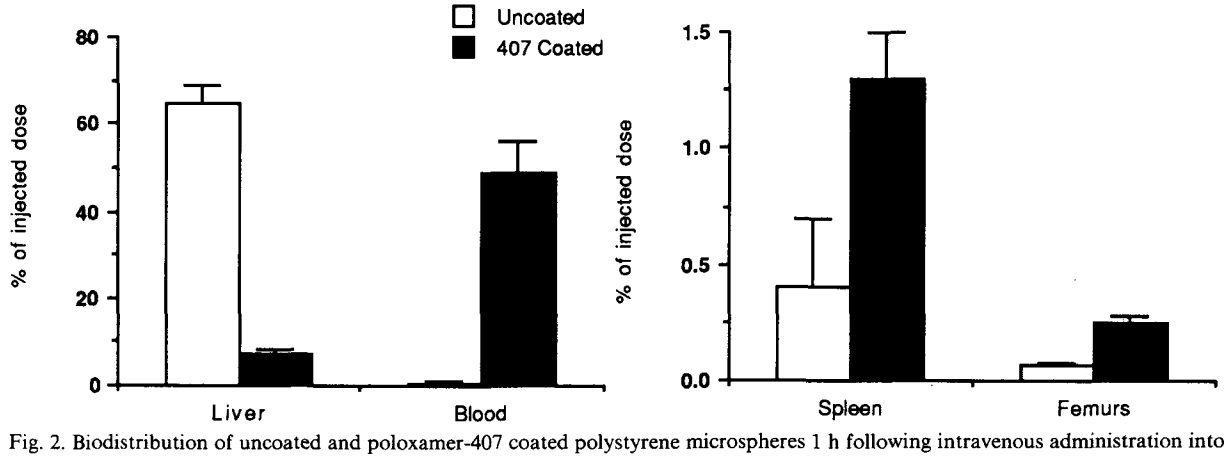

Fig. 2. Biodistribution of uncoated and poloxamer-407 coated polystyrene microspheres 1 h following intravenous administration into rats.

effect on the acyl chains of DSPC (Demel and Kruyff, 1976), provided no evidence of poloxamer penetration or adsorption onto the vesicles.

The inability of poloxamers to effectively coat liposomes is further supported by our in vivo studies, since no significant change in bone marrow retention or uptake by other organs of the reticuloenothelial system of intravenously injected poloxamer treated liposomes was found in comparison to control vesicles. These results are further supported by the unpublished observations of Senior and Gregoriadis (Senior, 1987) who found that pretreatment of liposomes, composed of either equimolar amounts of DSPC and Chol or PC and Chol, with poloxamers failed to produce any significant change from the usual clearance patterns of the respective uncoated vesicles following intravenous administration. These differences can not be attributed to species variation (rabbit/rat) since coating of polystyrene microspheres of similar size to liposomes prolonged their circulatory half-life and increased their femoral uptake by 3-fold.

Earlier Jamshaid et al. (1987) demonstrated that 30 min after intravenous administration into rabbits of ^{131}I-labelled phosphatidylcholine egg PC SUVs, 37% of the injected dose was associated with the liver and spleen regions, whereas precoating of vesicles with poloxamer resulted in recovery of 28% of the injected activity in this region as measured by gamma scintigraphy. This small difference was taken as evidence for poloxamer sorption on liposomes. Jamshaid et al. (1987) reasoned that, since the mean liver activity associated with blood pool is 26% of the circulating activity, the results for the coated vesicles represent only minimal uptake by the Kupffer cells and macrophages of the spleen. However, it should be considered that the assessment of liposome uptake by reticuloendothelial organs requires intact vesicles to be delivered and that the radiolabelled marker should remain with the vesicles throughout the course of the experiment. Extensive transfer of both radioactivity and mass of egg PC into high density lipoproteins (HDL) has now been well established (Scherphof et al., 1978; Tall, 1980). For example within a few minutes after intravenous administration of [^{14}C]phosphatidylcholine-labelled egg PC SUVs, HDL isolated from rat plasma had been enriched with approx. 60% of [^{14}C]PC (Tall, 1980). Thus, 60–70% of the activity remaining in circulation after intravenous administration of ^{131}I-labelled egg PC vesicles (Jamshaid et al., 1987) do not necessarily represent the activity associated with intact vesicles since the majority of the radiolabel marker and the mass of the phospholipid could be associated with HDLs. In contrast, our results are based on the clearance of [^{14}C]inulin-entrapped liposomes. Inulin is a reliable aqueous marker, since it is metabolically inert and is not taken up by tissues such as liver, spleen, and the bone marrow in free form (Patel et al., 1983). Further, any free inulin in circulation is rapidly excreted by kidneys. Thus, our results represent the fate of intact vesicles in blood and in association with the organs of the RES.

Furthermore, no indication of liposomal activity associated with spleen, lung, and bone marrow was given by Jamshaid et al. (1987) making the biodistribution of poloxamer coated liposomes difficult to assess. Finally, stability data from Jamshaid et al. (1988) suggest that the preincubation of egg PC vesicles in the presence of 0.01% (w/v) poloxamer is sufficient to release more than 70% of their entrapped aqueous marker, thus indicating that liposome integrity is not grossly retained even before contact with blood.

It is also possible to speculate that the phospholipid transfer to lipoproteins may, perhaps, occur at a faster rate from poloxamer treated than control egg PC liposomes which can further lead to underestimation of liposome uptake by RES.

In conclusion, pretreatment of stable liposomes with poloxamers under conditions described here and elsewhere (Jamshaid et al., 1988) does not redirect the vesicles effectively to the bone marrow or to any other sites within the body following intravenous administration. Grafting or covalent attachment of other polymeric systems, such as poly (ethylene glycol), to phospholipids such as phosphatidylethanolamine may provide an alternative approach to prevent liposome uptake by liver and redirect them more efficiently to other sites, such as the bone marrow, following intravenous administration.

Acknowledgement

We are grateful to SERC for the financial support.

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