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The polyoxyethylene/polyoxypropylene block co-polymer Poloxamer-407 selectively redirects intravenously injected microspheres to sinusoidal endothelial cells of rabbit bone marrow

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Small colloidal particulates (150 nm and below, in diameter) can be redirected specifically to the rabbit bone marrow following intravenous administration by coating their surface with the block co-polymer poloxamer-407, a non-ionic surfactant. The coated colloids are sequestered by the sinusoidal endothelial cells of the bone marrow and are accumulated in dense bodies within these cells. The uptake of poloxamer-407-coated colloids by marrow endothelial cells suggests that the steric repulsive barrier, imposed by the polyoxyethylene segment of the polymer, to particle–cell interaction can apparently be overcome by a specific interaction mechanism(s) with the cell surface. Such a dramatic uptake cannot be achieved with other block co-polymers of similar structure to poloxamer-407. The application of the current model for the site-specific targeting of drug carriers to bone marrow and the prevention of the adherence of metastases of tumours which selectively colonize the bone marrow endothelium is discussed.

Microsphere; Bone marrow; Endothelial cell; Poloxamer-407; Rabbit

1. INTRODUCTION

Unlike the sinusoidal system of the liver and spleen, the medullary sinusoidal system of the bone marrow is formed from an uninterrupted layer of spindle-shaped endothelial cells. The endothelial lining of the sinuses constitutes the major cellular component in the removal of particulate material from the circulation in the bone marrow [1]. Portions of the endothelial cells lining the marrow sinuses may be markedly attenuated, forming small fenestrae which are spanned by thin diaphragms. These randomly distributed diaphragmed fenestrae are also sites where plasma components and particulate materials (depending on their size) may permeate the endothelial lining [2,3].

The ability of the endothelial cells lining the marrow sinuses and the diaphragmed fenestrae to remove particulate matter from the blood stream raises the possibility of exploiting colloidal drug carriers for the delivery of therapeutic agents to this organ [4]. These may include anti-infective agents for the treatment of endothelial cell infections (such as Rickettsia); scanning agents for diagnostic imaging; cyclosporin-A for preventing rejection following organ transplantation and haematopoietic growth factors for self-renewal, proliferation, maturation, functional integrity and activation of marrow cells during intensive chemotherapy of cancer and retroviral diseases [4].

However, following intravenous administration the majority of particulate colloids will be sequestered by the mononuclear phagocytes of the liver and to a lesser extent of those of the spleen [4]. This process thus limits the use of colloidal drug carrier systems for efficient delivery of therapeutic agents to the bone marrow. In order to redirect particles to the bone marrow it is necessary to minimize or prevent their interaction with the reticuloendothelial elements of the liver and the spleen. Recently, we demonstrated that in rabbits the efficient sequestration of small colloids (60 nm in diameter) from the blood by liver and spleen can be reduced dramatically by coating their surface with two block co-polymers of the poloxamer series: poloxamer-338 (weight average polyethylene glycol (PEG)/polyethylene oxide (PEO) equivalent molecular mass: 13,850) and poloxamer-407 (weight average PEG/PEO equivalent molecular mass: 13,310) [5,6]. This effect is believed to be due to the steric repulsive effect generated by the hydrophilic polyoxyethylene segments of these poloxamers both to protein adsorption and particle–macrophage adhesion [7]. Remarkably, these polymers redirected a significant portion of microspheres to the bone marrow and of the two poloxamer-407 was extremely effective, resulting in the deposition of about 50% of the injected dose of microspheres in the bone marrow of rabbits [5,6]. We have now studied the site of localization of poloxamer-407-coated latex microspheres of various sizes in the rabbit bone marrow.

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2. MATERIALS AND METHODS

Polystyrene microspheres of various sizes, 60, 150 and 250 nm in diameter (Polysciences, UK) were surface-labelled with Na[^{131}I] (Amersham, UK) as described earlier [5–7]. Polystyrene microspheres were coated with poloxamer-407 (Ugine Kuhlman, UK) by preincubation in a 2.0% w/v solution at room temperature overnight. The adsorption of polymer onto the surface of microspheres was confirmed by measuring the surface layer thickness and surface potential of the particles by means of photon correlation spectroscopy and laser Doppler anemometry, respectively, as previously described [5–7]. The quantities of the adsorbed poloxamer-407 onto the surface of microspheres were measured from the adsorption isotherms [8]. Groups of three male New Zealand White rabbits, 2.50 ± 0.25 kg, were injected intravenously via the marginal ear vein with either 4.0 mg of uncoated or poloxamer-407-coated microspheres. Blood samples were taken at suitable intervals and the activity measured. The distribution of microspheres in the body was followed by gamma scintigraphy. Dynamic and static images of the liver and spleen regions and the left hind limb were analysed [5,6]. Animals were sacrificed 24 h post administration and the activity in selective organs was determined using a large sample volume gamma counter.

Bone marrow was extracted and fixed by the method of De Bruyn et al. [9]. Immersion rather than perfusion fixation was performed since the former technique is better in preserving the intravascular distribution of the tracer particles and, consequently, provides more reliable images. Briefly marrow was immersed in the fixative (2.5% glutaraldehyde in 0.08 M cacodylate buffer, pH 7.4) for 4 h. After initial aldehyde fixation the marrow samples were further processed in the conventional manner: rinsed in buffer, postfixed in 1% (v/v) osmium tetroxide in distilled water, dehydrated with graded ethanols, embedded in EMIX resin (Fison, UK), trimmed and cut into 80-nm sections, and stained with uranyl acetate followed by lead citrate. Micrographs were taken with an JEOL 1200 EX electron microscope operated at 80 kV.

3. RESULTS AND DISCUSSION

The results in Fig. 1 and Table I demonstrate the effect of microsphere size and poloxamer coating on particulate sequestration by the bone marrow. Hepatic uptake of small microspheres (60 and 150 nm in diameter) is greatly suppressed when their surface is coated with poloxamer-407. The coated microspheres remain in the systemic circulation for up to 7–8 h post administration, but eventually the majority of the dose is redirected to the bone marrow and the process is complete at 24 h post administration as determined by scintigraphy and organ biodistribution studies. The coated particles are dispersed throughout the major parts of the skeleton and are presumably associated with the bone marrow (Fig. 2). For larger coated particles (250 nm in diameter) the liver and spleen still constitute the major sites of clearance and as a result a smaller fraction of coated particles reach the marrow.

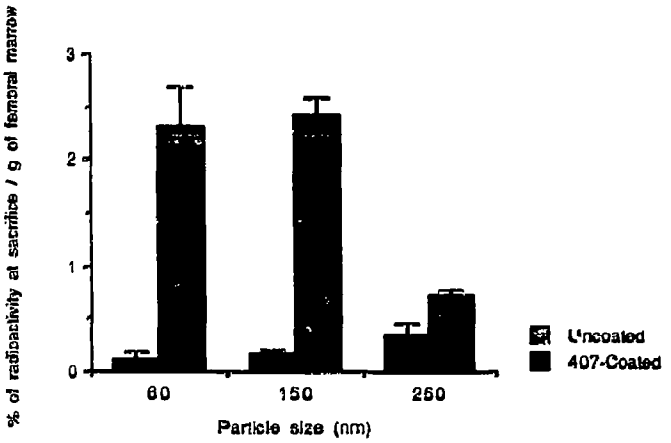


Fig. 1. Association of microspheres of various sizes with femoral bone marrow at sacrifice (24 h post administration).

Adsorption isotherm studies have shown that the plateau concentration of poloxamer-407 adsorbed to the surface of 60-nm particles is lower than that determined on 250-nm particles (Table II). This may suggest that the surfactant adsorbs to the smaller particles in a less crowded arrangement than to particles of a larger diameter and smaller surface curvature [10]. The reduction in polymer chain mobility produced by the adsorption of poloxamer to larger as opposed to smaller particles may lead to an attenuation of the effectiveness of the steric barrier and thus explain the increase in hepatic and splenic sequestration of larger particles in spite of the greater quantity of poloxamer adsorbed to their surface [10].

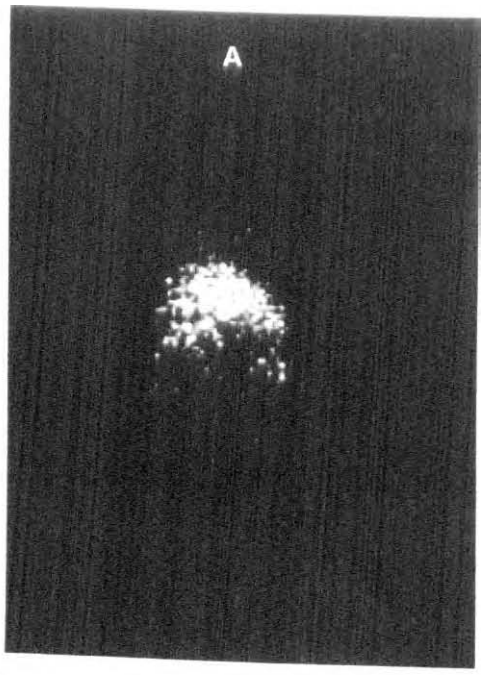
Electron microscopic studies of thin sections of femoral marrow demonstrated that poloxamer-407-coated microspheres (150 nm in diameter) were exclusively as-

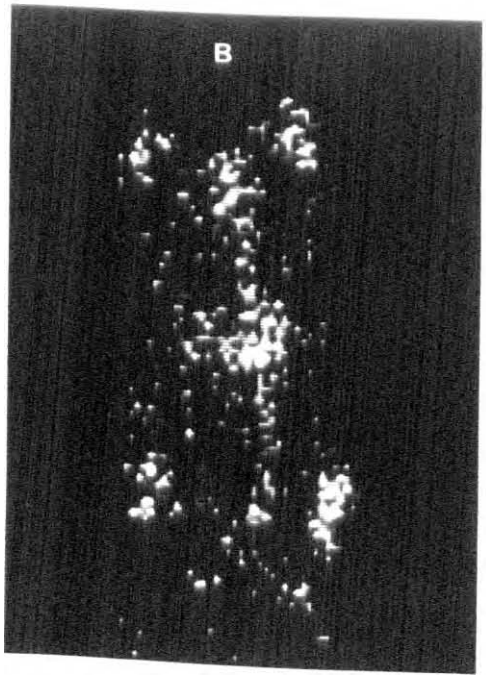
Table I

Biodistribution of ^{131}I-labelled latex microspheres 24 h following intravenous administration into rabbits

	% of total radioactivity recovered at sacrifice									
	Liver		Spleen		Femur		Blood		Carcass	
Microspheres (nm)	U*	C**	U	C	U	C	U	C	U	C
60	66.0 ± 1.5	15.8 ± 4.5	4.1 ± 0.9	2.0 ± 0.9	0.8 ± 0.2	4.9 ± 1.4	2.0 ± 1.0	5.1 ± 1.3	36.5 ± 6.5	74.4 ± 5.6
150	81.9 ± 8.2	18.7 ± 4.5	5.5 ± 3.7	3.9 ± 2.8	0.5 ± 0.2	5.8 ± 2.3	2.3 ± 0.8	2.3 ± 0.8	10.0 ± 4.6	76.1 ± 9.3
250	75.4 ± 5.9	51.7 ± 8.7	7.1 ± 1.9	9.8 ± 3.1	0.5 ± 0.2	1.3 ± 0.2	1.5 ± 0.1	1.5 ± 0.3	15.5 ± 4.3	36.4 ± 11.7

*Uncoated microspheres; **poloxamer-407-coated microspheres. Carcass represents total activity associated with bones (including marrow), muscles and skin.





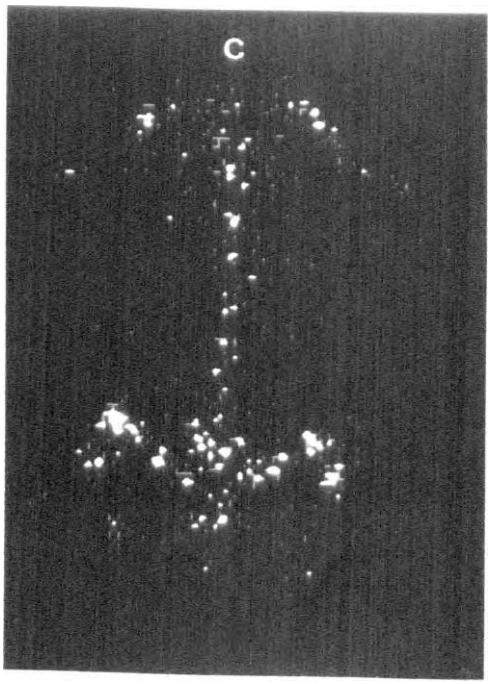


Fig. 2. Scintiscans of rabbits 24 h after intravenous administration of uncoated and polyxamer-407 coated polystyrene microspheres (150 nm). (A) Uncoated microspheres, approximately 90% of the radioactivity was deposited in liver and spleen and the half-life for clearance was of the order of 1 min. (B) Poloxamer-407-coated microspheres, approximately 25% of the radioactivity was taken up rapidly by the liver and spleen; diversion of particles from the liver and spleen region to skeleton and soft tissue is apparent. (C) Carcass (represents bones including the marrow, muscles and the skin) of the same animal as (B), radioactivity associated with skeleton is clearly apparent. The level of radioactivity associated with each hind limb is approximately 20%, pelvis 15%, vertebral column 25%, forelimbs and skull 20% of the total activity of the skeleton.

sociated with the endothelial cells of the sinus wall at 24 h post administration (Fig. 3). Groups of latex microspheres were sequestered within large spherical vesicles (ranging from 0.5 to 1.2 μm in diameter) in cytoplasm of sinusoidal endothelial cells. These vesicles contain an electron-opaque matter and their morphology resembles the 'dense-bodies' described by De Bruyn et al. [11] and others [12]. While sinusoidal endothelium is vigorously active in the uptake process, the endothelium of other marrow vessels remained indifferent to the presence of intravascular poloxamer-407-coated colloids as determined by electron microscopy.

In the rabbit (unlike species such as the rat, dog and guinea pig) marrow macrophages penetrate the endothelium by sending cytoplasmic processes into the lumen and in doing so, have the ability to monitor the circulation and remove particulate materials from the blood [13]. These macrophages are known as perisinusoidal phagocytes [13]. Electron microscopic studies further demonstrated the inability of perisinusoidal macrophages as well as other marrow granular (granulocytes, lymphocytes and reticular cells) to ingest poloxamer-407-coated microspheres. Fluorescence-activated cell sorting studies, using fluorescent polystyrene microspheres (Polysciences, UK) coated with poloxamer-407, also confirmed the inability of marrow macrophages and granular cells to interact with poloxamer-407-coated microspheres (data not shown). These observations suggest that the steric barrier generated by the hydrophilic moiety of the poloxamer-407 also prevents phagocytosis of microspheres by perisinusoidal macrophages [7].

It should be emphasized that the ability of polo-

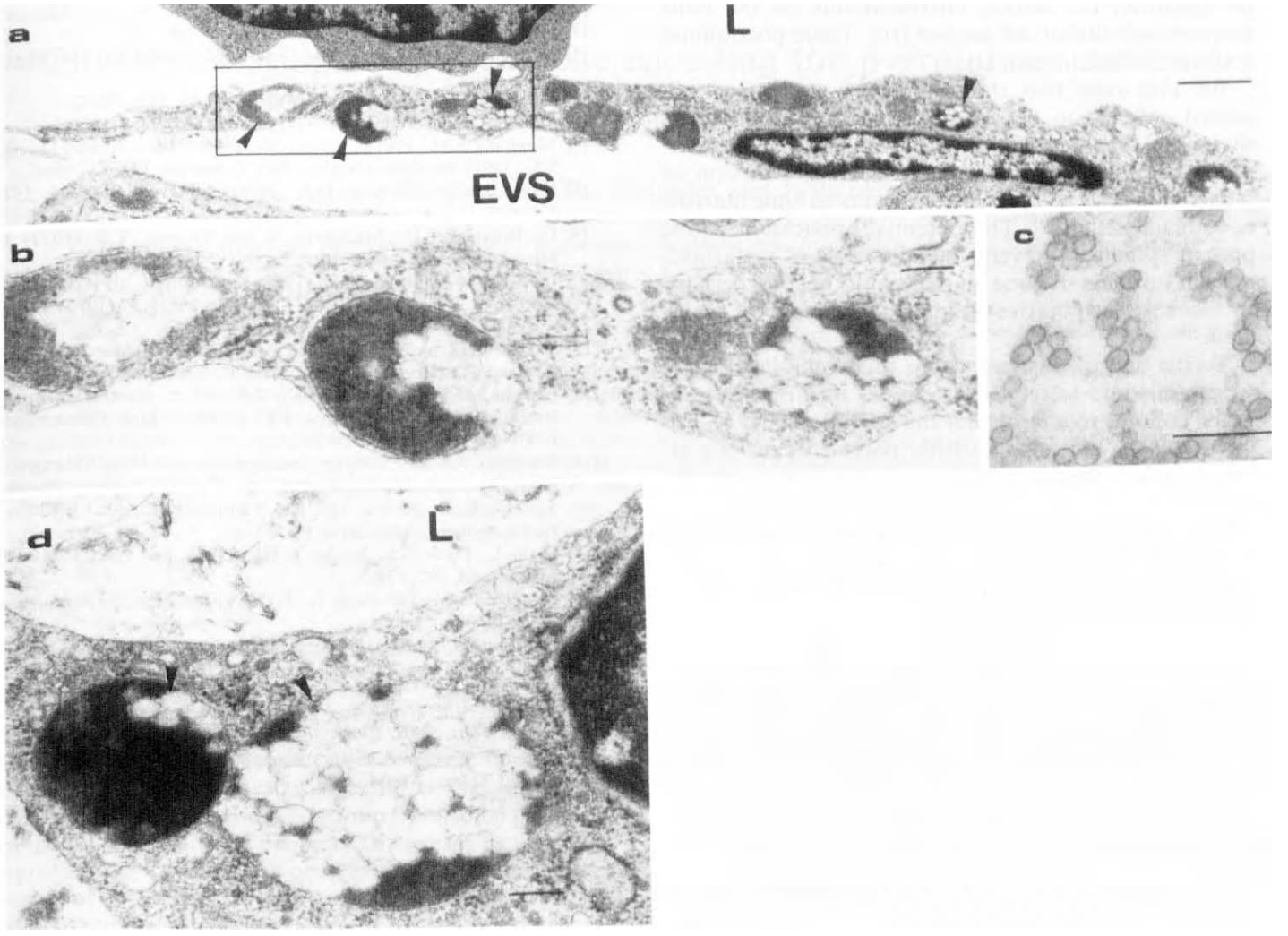


Fig. 3. Electron micrographs showing the localization of poloxamer-coated latex-microspheres (150 nm) in sinusoidal endothelial cells 24 h after intravenous administration. (a) Poloxamer-407-coated microspheres are present in large vesicles designated as dense bodies (arrows); L, lumen of the sinusoids; EVS, extravascular space; bar = 1 μm. (b) Magnified view of the box region in (a), bar = 200 nm. (c) Electron micrographs of latex microspheres (150 nm) incubated in plasma, bar = 500 nm. (d) Section through a sinusoidal endothelial cell showing accumulation of poloxamer-coated microspheres in a large dense body, bar = 200 nm.

xamer-407 to redirect small-sized microspheres to the bone marrow is not due to the 'spill-over' phenomenon [14] since other members of block co-polymers of similar structure to poloxamer-407 (poloxamer-238, poloxamines-908, -1504 and -1508) failed to redirect microspheres to the bone marrow. These coating agents

Table II

Quantities of the polymer adsorbed onto the surface of microspheres

Microsphere size (nm)	Adsorbed polymer (mol/m^2)	Surface density (nm^2 polystyrene/mol)
60	14×10^{-8}	12
150	21×10^{-8}	8
250	29×10^{-8}	6

effectively minimize the capture of small-sized particles (60 nm in diameter) by both macrophages of the liver and the spleen and, consequently, keep microspheres within the vascular system even up to 8-days post administration [15]. The ability of sinusoidal endothelial cells of the bone marrow to interact with poloxamer-407-coated microspheres is therefore remarkable. In doing so, the steric repulsive barrier to particle–cell interaction imposed by the polyoxyethylene segment of poloxamer-407 on the surface of colloids can apparently be overcome. It is not clear at present whether this recognition is receptor mediated. The recognition may be mediated via a plasma component (such as erythropoietin, transferrin, transcobalamin, etc.) or an endothelially derived factor which specifically adsorbs onto the surface of poloxamer-407-coated colloids and exhib-

its specificity for certain microdomains on the bone marrow endothelial cell surface [16]. These possibilities warrant further investigation.

We also note that the homing of poloxamer-407-coated colloids to bone marrow also resembles that shown by organ-specific metastases. This is best compared with the selective and site-specific adhesion of breast, follicular and prostatic tumours to bone marrow endothelial cells [17]. The potential application of the present system to prevent the adherence of metastases of such tumours to bone marrow endothelium, perhaps by blocking the putative receptors, remains to be evaluated.

To the best of our knowledge this is the first report to demonstrate selective delivery of intravenously injected colloids to a particular line of endothelial cells in vivo with the aid of a synthetic polymer. Previous attempts in site-specific targeting to endothelial cells exploit the use of expensive neoglycoproteins and monoclonal antibodies [18–20]. The ability of poloxamer-407 to redirect biodegradable drug carriers such as poly (lactide-co-glycolide) microspheres to the bone marrow is currently under investigation.

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