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Differences in the molecular weight profile of poloxamer 407 affect its ability to redirect intravenously administered colloids to the bone marrow

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Summary

Samples of poloxamer 407 obtained from different suppliers have been assessed for their ability to redirect model polystyrene colloids to the bone marrow in rabbits. Only poloxamer 407 obtained from one supplier was found to produce the targeting effect seen previously. The variation in the biodistribution patterns produced by the different samples of poloxamer 407 may by caused by differences in the molecular weight distribution of the polymer.

Recently, a number of investigators have shown that by modifying the surface of colloidal drug delivery systems with poly(ethylene oxide)-co-(propylene oxide) polymers of the poloxamer series, their interaction with the biological milieu can be significantly altered (Illum and Davis, 1984, 1987; Illum et al., 1989; Troster et al., 1990; Moghimi et al., 1992). These studies have identified poloxamer 407 as a coating agent capable of redirecting a substantial proportion of the dose of an injected colloid to the bone marrow in rabbits (Illum and Davis, 1987). In this communication, we report a discrepancy in the bone marrow targeting properties of poloxamer 407 obtained from different suppliers and an attempt to elucidate the differences in physicochemical characteristics responsible for this phenomenon.

Samples of poloxamer 407 were obtained from Eugine Kuhlman, U.K. (polymer A); ICI, France (polymer B) and BASF-Wyandotte, U.S.A. (Polymer C) and were used as received. The molecular weights of these samples were determined by the method of raised temperature gel-permeation chromatography. Briefly, samples of poloxamer 407 were dissolved in dimethylformamide (2% w/v) and filtered through a 0.2 μm polyamide membrane prior to the chromatography. Sample solutions were passed through two columns (30 cm in length) of Polymer Laboratory Mixed Gel, 10.0 μm (RAPRA Technology, U.K.) at a flow rate of 1.0 ml/min at 80°C. The eluent was monitored by measuring the refractive index. The system was calibrated with polyethylene glycol (PEG) and polyethylene oxide (PEO) standards

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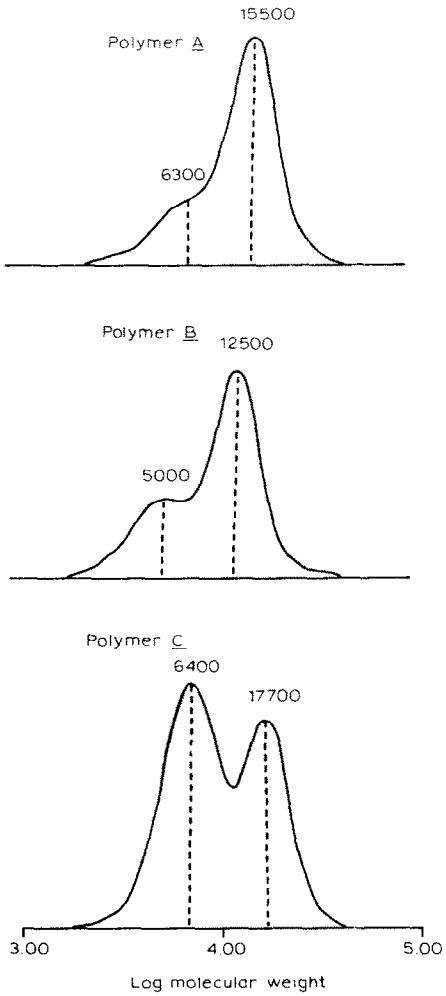

Fig. 1. Molecular weight profiles of poloxamer 407 obtained from different suppliers. The molecular weights of the constituent peaks are shown. The x-axis is refractive index (arbitrary units).

and the results are therefore expressed as the 'PEG/PEO equivalent' molecular masses. The ethylene oxide content of the polymers was measured by NMR spectroscopy (Brucker 360 MHz) in deuterated chloroform. The mol% content of ethylene oxide was determined by a comparison of the integration from the methyl peak (generated from polypropylene oxide segment of the molecule) to the total ethylene oxide/propylene oxide signal.

The adsorbed layer thickness produced by the adsorption of poloxamer 407 to the surface of 60 nm polystyrene microspheres (Polysciences, U.K.) was determined by photon correlation spectroscopy and laser doppler anemometry (Malvern Instruments, type LGK-7626 and Zetasizer 4, respectively) as described in detail elsewhere (Illum and Davis, 1987).

For the in vivo studies, latex micospheres (60 nm in diameter) were surface labelled with $Na^{131}I$ (Amersham, U.K.) and coated with poloxamer as described previously (Illum and Davis, 1987). Poloxamer 407 coated and uncoated labelled microspheres (0.4 ml of a 1% w/v suspension) were administered to pairs of male New Zealand White rabbits (2.5 ± 0.2 kg) via the marginal ear vein. Animals were killed 24 h post-administration and the activity associated with the organs of the reticuloendothelial system, the blood and the carcass measured.

The elution profiles in Fig. 1 and the data in Table 1 demonstrate considerable differences in the physicochemical properties of poloxamer 407 samples obtained from the three different sources. The elution profiles of each of the polymers

TABLE 1

Some physicochemical characteristics of poloxamer 407 from various sources

Poloxamer 407	Source	PEG/PEO equivalent molecular weight	Ethylene oxide content (mol%)	Adsorbed layer thickness (Å) [a]	Zeta potential (mV) [b]
A	Eugine Kuhlman, U.K.	13310	84.6	168	−3.3
B	ICI, France	10400	76.2	199	−4.6
C	BASF, U.S.A.	11540	80.8	164	−2.4

[a] Represents the thickness of the adsorbed polymer on the surface of 60 nm polystyrene microspheres.

[b] Represents the zeta potential of 60 nm polystyrene microspheres coated with an adsorbed layer of polymer at pH 7.4 in 1 mM phosphate buffer (zeta potential of uncoated microspheres = −87.5 mV).

TABLE 2

Biodistribution of uncoated and polymeric coated microspheres 24 h following intravenous administration into rabbits

Microspheres	% of in vivo radioactivity [a]				
	Liver	Spleen	Femur	Blood	Carcass [b]
Uncoated	74.5/64.5	5.3/5.7	0.5/0.5	3.8/5.0	15.9/24.2
Polymer A-coated	18.9/9.9	0.9/1.1	4.7/6.4	2.7/5.1	68.6/75.2
Polymer B-coated	26.4/44.2	1.5/1.4	1.1/0.9	29.3/14.7	41.7/38.9
Polymer C-coated	41.9/39.7	1.5/0.9	0.7/0.6	23.0/22.7	32.9/36.1

[a] Values for individual rabbits are given.

[b] Carcass represents activity associated with bones (including marrow), muscles and skin.

demonstrated a bimodal distribution, although in the case of polymer A, the secondary peak was very minor. The weight average molecular weights are listed in Table 1 and the molecular weights of the individual peaks are marked on Fig 1. In addition to the differences in molecular weight these polymers exhibited differences in their ethylene oxide contents, although these were not consistent with the associated variation in adsorbed layer thickness and zeta potential produced on 60 nm polystyrene microspheres (Table 1). Nevertheless, all the polymers produced thick adsorbed layers on polystyrene microspheres and considerably reduced the zeta potential associated with uncoated microspheres, suggesting the presence of an effective steric barrier.

The results in Table 2 demonstrate the in vivo distribution in rabbits of uncoated microspheres and microspheres coated with different samples of poloxamer 407. All three sources of poloxamer 407 were able to decrease both hepatic and splenic sequestration of microspheres in comparison to uncoated microspheres, although polymer A was most effective in this respect. Polymer A was subsequently found to redirect a considerable proportion of the microspheres to the bone marrow, as defined by the percentage activity associated with one femur, 24 h post-administration (Table 2). This is in accord with the previous published observations of Illum and Davis (1987). In contrast, polymers B and C failed to produce a significant bone marrow targeting effect. A higher proportion of the microspheres coated with polymers B and C remained in the systemic circulation at 24 h post-dose compared to control and microspheres coated with polymer A.

In order that the possible role of the constituent peaks of the bimodally distributed polymers, on the bone marrow targeting effect could be examined, an attempt was made to separate the two constituent peaks in polymer C by gel permeation chromatography (polymer C was chosen due to the larger degree of separation between its constituent peaks). However, the separated components, after adsorption onto 60 nm polystyrene microspheres, also failed to redirect microspheres to the bone marrow (data not shown).

Recent electron microscopic studies have demonstrated that polystyrene microspheres coated with polymer A are specifically redirected to the sinusoidal endothelial lining cells of the rabbit bone marrow after intravenous administration (manuscript in preparation). This specific recognition may be mediated through direct interaction of poloxamer 407 on the microsphere surface (or an associated plasma component) with certain classes of lumenal plasmalemma receptors on the sinusoidal endothelial cells. In this respect, we believe the surface density and conformation of poloxamer 407 on the surface of microspheres could play an important role in this recognition, since the targeting effect cannot be observed with others of the poloxamer series, except to a lesser extent, poloxamer 338 (Illum and Davis, 1984; Moghimi et al., 1991). The density and conformation of polymers B and C are presumably different from those of polymer A, since they exhibit different physicochemical characteristics (i.e., in terms of molecular weight, ethylene oxide content and adsorbed layer thickness).

The conformation of the steric barrier produced by a polymer of a certain average molecular weight and ethylene oxide content, but with a bimodal molecular weight distribution (such as that exhibited by polymers B and C), is likely to be different from that produced by a polymer with very similar physical characteristics, but exhibiting a monomodal molecular weight distribution (polymer A). Therefore, it can be suggested that the presence of the two molecular weight subgroups in polymers B and C affects the configuration and conformation displayed by the steric barrier on the surface of microspheres such that it may not attract a putative targeting plasma component or favour the interaction of microspheres with plasmalemma receptors on sinusoidal endothelial cells present in the bone marrow.

In contrast to what has been shown in the rabbit model, both polymers A and C can modify the proportion of microspheres redirected to the rat femur as compared to control. For example, the activity associated with a rat femur was 0.05 ± 0.01, 0.26 ± 0.10 and $0.22 \pm 0.01\%$ of administered dose for uncoated, polymer A- and polymer C-coated microspheres, respectively. Electron microscopic studies are necessary to demonstrate whether coated microspheres are associated with rat bone marrow sinusoidal endothelial cells or trapped in sinuses.

This study has demonstrated a batch-related biodistribution pattern for poloxamer 407-coated microspheres which, it appears, may be caused by differences in the molecular weight distribution of the polymer. However, further analysis of the surface properties and conformation of poloxamer 407 on the surface of these systems is now required. This information may lead to the elucidation of the specific characteristics required by colloidal delivery systems sterically stabilized by PEG in order to promote redirection of microspheres to the bone marrow.

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