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The effect of adsorbed poloxamer 188 and 407 surfactants on the intestinal uptake of 60-nm polystyrene particles after oral administration in the rat

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Abstract

The surface properties of 60-nm polystyrene particles were modified by the adsorption of poloxamer 188 and 407 surfactants, and the effect of this change on the intestinal uptake of the particles studied. In comparison to uncoated polystyrene particles, the surfactant-coated particles had an increased particle diameter, as measured by photon correlation spectrometry, and lower zeta potential. Uptake of the coated polystyrene particles by gut epithelial tissue was studied in female Sprague–Dawley rats (180 g, 9 weeks old) after 5 days oral dosing by gavage. The small and large intestine was divided into lymphoid and non-lymphoid tissue, prior to analysis for polystyrene by gel permeation chromatography and visualization of particle uptake using fluorescent microscopy. The adsorption of poloxamer surfactants onto the polystyrene particles appeared to completely inhibit particle uptake in the small intestine. Polystyrene uptake was shunted to the large intestine, with the detection in this region of approximately 3% and 1.5% of the dose of poloxamer 407 and 188 coated particles, respectively. This uptake was less than the 10% uptake across the entire GI tract (5.1% uptake in the large intestine) determined for uncoated polystyrene particles.

Keywords: Oral drug delivery; Polystyrene nanoparticles; Poloxamer surfactants; Adsorption isotherms; Particulate uptake

1. Introduction

Attack by enzymes, instability in the gut, and the barriers to absorption and transport, must be surmounted in order to achieve the oral delivery of labile drug molecules such as therapeutic peptides and proteins. Considerable progress in the understanding of the use of carriers to protect and deliver labile drugs has been made, with much attention focused recently on the possibility of employing nano- and micro-particulates as oral carriers, exploiting the phenomenon of particulate uptake from the GI tract by the Gut Associated Lymphoid Tissue (GALT) or other intestinal sites

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(O'Hagan, 1990; Kreuter, 1991; Florence and Jani, 1993).

Research into oral particulate uptake has not been without controversy; the phenomenon of particulate uptake as a useful process has been questioned (O'Mullane et al., 1987), but research in many laboratories has repeatedly demonstrated the phenomenon, suggesting that a paradigm shift is required in our current understanding of the processes involved in oral absorption. Obviously further information on the oral absorption of particles is required, not least to determine if the phenomenon occurs to a sufficient extent to be useful for drug delivery. A more thorough understanding of the processes involved in the uptake of particles would also be advantageous for the rational design of a particulate carrier with optimal properties for uptake.

Much work on the phenomenon of oral particulate uptake has been undertaken with non-degradable polystyrene particles. This paper adds to this understanding of polystyrene uptake and describes how the oral uptake of polystyrene particles is affected by adsorbed poloxamer surfactants, which converts the hydrophobic surface to a hydrophilic one. The poloxamers are non-ionic block co-polymeric surfactants with a central, hydrophobic polyoxypropylene (POP) portion, flanked by two hydrophilic polyoxyethylene (POE) chains which protrude into the dispersion medium. Poloxamers 188 and 407 (Table 1) were selected in this work for adsorption to polystyrene particles. We report here a shift in the site of uptake and absorption from the small intestine to the large intestine, as a result of poloxamer treatment of the particles.

2. Materials and methods

2.1. Nanoparticles

Monodisperse non-ionized polystyrene nanoparticles with covalently linked fluorescein (59 ± 3 nm, 2.5% solids-latex) were obtained from Polysciences Ltd. (Northampton, UK).

Nanoparticles with adsorbed poloxamer surfactant were prepared by mixing a 1% solution of poloxamer 188 or 407 (Pluronic F68™ or Pluronic F127™, respectively, obtained from ICI, Cheshire, UK) with a polystyrene latex (59 nm) dispersion and shaking the dispersions for 24 h at 37°C.

2.2. Adsorption isotherms: poloxamers on polystyrene

Adsorption isotherms for poloxamers on polystyrene were prepared according to the method of Kayes and Rawlins (1979). Various concentrations (from 0.01% to 4%) of poloxamer 188 and 407 were prepared in triplicate and shaken with polystyrene microspheres (59 nm) at 37°C for 24 h, to allow adsorption to occur. The dispersions were centrifuged at 200 000 g for 1.5 h in a Sorvall Ultra 80 Combiplus centrifuge (Dupont, Herts, UK) to remove the latex. The equilibrium concentration of the supernatant was measured, after suitable dilution, from standard curves of absorbance versus poloxamer concentration, prepared according to Baleux (1972). Adsorption isotherms were constructed, plotting the amount of poloxamer adsorbed (μmole/m^2) against the equilibrium concentration of poloxamer. The surface area (m^2) of the polystyrene microspheres available for adsorption was calculated from the surface area of a sphere and the number of particles per ml in the latex suspension, where:

$$\text{the number of particles per ml} = \frac{6W \times 10^{12}}{\rho\pi\phi^3}$$

and W = grams polymer per ml in latex; ρ = density of polymer in grams per ml (1.05 for polystyrene); ϕ = diameter in μm of latex particles.

Table 1
Properties of poloxamer 188 and 407

Poloxamer	Average M_r	a	b	M_r POP[a]	% EO[b]
188	8350	75	30	1750	80
407	12 000	98	67	4000	70

a, number of POP chains; b, number of POE chains.
[a]Molecular weight of POP.
[b]Approximate weight percentage of POE.

2.3. Particle characterization

Particle sizing of surfactant-coated and uncoated polystyrene nanoparticles was carried out by photon correlation spectrometry (PCS) using a Multi 8 Computing Correlator Type 7032CE with Liconix He/Cd Laser (Malvern, UK). The size of surfactant-coated nanoparticles was determined at weekly intervals for a period of 1 month. Electrophoretic mobility (EM) was determined using a Malvern Zetameter (Simpson Model 3326, Malvern, UK). Particles of 59 nm diameter were too small to be tracked directly, therefore coated and uncoated 500-nm particles, in 0.01 N KCl, were studied. Measurements were performed at 25°C and 67 V, at a tracking length of 120 μm. The EM of the particles was determined from the velocity of the particles in the applied electric field by:

$$EM = \frac{\text{Particle Velocity (nm/s)}}{\text{Field Strength (V/cm)}}$$

The zeta potential (ZP) was calculated from the EM using the Helmholtz–Smoluchowski formula:

$$ZP = \frac{EM.4\pi V_t}{D_t}$$

where V_t = Viscosity and D_t = Dielectric Constant.

The EM and ZP potential of surfactant-coated particles was determined at weekly intervals for a period of 1 month.

2.4. Animals, dosing and sample collection

Female Sprague–Dawley rats (average weight 180 g, 9 weeks old) were maintained in metabolic cages and orally dosed via gavage as described previously (Hillery et al., 1994). Animals were dosed each morning for a period of 5 days with 0.1 ml of either: (i) saline, (ii) uncoated polystyrene particles, (iii) poloxamer 188 coated polystyrene particles and (iv) poloxamer 407 coated polystyrene particles. After the final dose, the animals were fasted for 12 h to clear the gut of food and unabsorbed microspheres and then killed. Sample collection was carried out according to the method of Hillery et al. (1994), with the stomach, small intestine with adhering mesenteric connective tissue, mesenteric node and large intestine taken for analysis; these gut tissues were washed carefully and processed for Gel Permeation Chromatography (GPC) analysis as described previously (Jani et al., 1990).

2.5. Quantitation of polystyrene uptake

The GI tract was divided into sections by careful dissection: the Lymphoid Small Intestine (LSI), Non-Lymphoid Small Intestine (NLSI), Lymphoid Large Intestine (LLI) and Non-Lymphoid Large Intestine (NLLI), as described previously (Hillery et al., 1994). The respective gut sections were analysed for polystyrene content using GPC and results were adjusted to account for 70% recovery and detection sensitivity.

2.6. Histology

Frozen sections of the various gut regions were prepared using a cryostat and the particles viewed with a fluorescent microscope (Nikon Microphot; Nikon, Teleford, UK) at fluorescein isothiocyanate filter setting (λ = 340 nm).

3. Results and discussion

3.1. Characterization of poloxamer-coated polystyrene particles

3.1.1. Adsorption isotherms

Langmurian isotherms were obtained for poloxamer 188 and 407 on polystyrene microspheres (Fig. 1), and the area occupied per molecule of poloxamer at the polystyrene/water interface under equilibrium conditions was determined. Poloxamer 188 occupied 12.8 nm^2 at the polystyrene/water interface, which was in agreement with previous determinations (Hillson, 1963; Kayes and Rawlins, 1979), whereas the determination of 5.9 nm^2 occupied by poloxamer 407 was less than that of 11.9 nm^2 obtained by Porter et al. (1992).

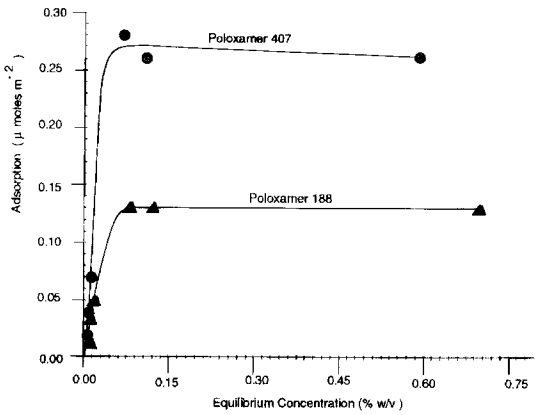

Fig. 1. Adsorption isotherms of poloxamer 188 and 407 on polystyrene latex particles (59 nm).

Although poloxamer 407 possesses a larger POP unit than poloxamer 188, it occupied a smaller area at the interface than poloxamer 188, perhaps because the POP region of poloxamer 407 formed small loops or was tightly coiled on the surface of the polystyrene, as was reported for various poloxamer surfactants at the polystyrene/water interface (Kayes and Rawlins, 1979) and the air/water interface (Prasad et al., 1979). Furthermore, poloxamer 407 associates into micelles having a mean aggregate number of 40 at 40°C (Rassing and Attwood, 1983) and this micellar association may have affected its adsorption behaviour, as these adsorption experiments were carried out at 37°C.

The plateau region of the adsorption isotherm was reached when polystyrene microspheres were incubated with 0.1% solutions of the poloxamers (Fig. 1), and the particles were incubated with 1% solutions for in vivo dosing experiments.

3.1.2. Surface characterization

Adsorption of the poloxamer surfactants to the surface of the polystyrene particles resulted in an increase in the apparent particle diameter, as measured by PCS (Table 2). Poloxamer 407 (67 ethylene oxide units) caused an increase in the particle diameter to 72 nm. The adsorption of poloxamer 188 (30 ethylene oxide units) increased the diameter to 65 nm, in agreement with the expectation that the thickness of the adsorbed poloxamer coating was proportional to the number of ethylene oxide units (Illum et al., 1987b).

Zeta potential was reduced by adsorption of the poloxamers, the thicker adsorbed layer of poloxamer 407 shifting the plane of shear further from the particle surface and reducing the zeta potential from -30 mV to -14 mV (Table 2).

These results provide clear in vitro evidence that the surface characteristics of the polystyrene particles were modified subsequent to incubation, and the data demonstrate the stability of the adsorbed coating, which was not desorbed into either water or electrolyte solutions over a 1-month period.

3.2. Oral uptake of poloxamer-coated polystyrene particles

Adsorbed poloxamers appear to block the uptake of 60-nm polystyrene particles in the small intestine of the rat GI tract (Fig. 2). Particle uptake was shunted to the large intestine, where only 3% of the administered dose of poloxamer 407 coated particles and 1.5% of poloxamer 188 coated particles was taken up, in contrast to the previously reported uptake of 10% across the entire GI tract for uncoated particles, with 5.1% of this uptake occurring in the large intestine (Hillery et al., 1994). The results obtained using GPC corroborated with the visual evidence obtained using fluorescent microscopy. No particles were observed in the small intestinal sections of animals dosed with surfactant-coated particles, and the number of particles observed in the large intestinal sections was significantly reduced in comparison to uncoated particles. This work provides further proof, not only that particulate uptake occurs, but that it is possible to regulate and control the process.

The surfactants sodium lauryl sulphate, polyoxyethylene-9-lauryl ether, and oleic and monoolein fatty acids can alter membrane lipid organization and typically enhance transcellular transport (Hirai et al., 1981; Muranushi et al., 1981; Sakai et al., 1986); surfactants may also function as absorption enhancers by solubilizing the mucosal barrier. It has been postulated that the use of poloxamer surfactants may enhance the

Table 2
Surface characterization of surfactant-coated and uncoated polystyrene particles

Coating agent	z average diameter (nm) ± S.D.	Adsorbed layer thickness (nm)	Zeta potential (mV) ± S.D.
None	56.3 ± 1.2	0	−30 ± 2.5
Poloxamer 407	72.4 ± 1.0	16	−14 ± 3.8
Poloxamer 188	65.3 ± 1.1	9	−19 ± 2.5

oral uptake of particles, because these surfactants can cause damage to the gastric mucosa (O'Hagan, 1990). However, the opposite effect was observed here, with an inhibition of uptake demonstrated. The poloxamer surfactants have a low toxicity profile and are licensed for use in food and drug formulations (Schmolka, 1991), suggesting that the type of damage inflicted by other surfactants on the integrity of the gut mucosa does not occur with the poloxamers. No histological damage to the gastric mucosa was observed in this work.

The adsorbed surfactant layer modified the surface properties of the polystyrene particles from hydrophobic to hydrophilic; the reduced uptake

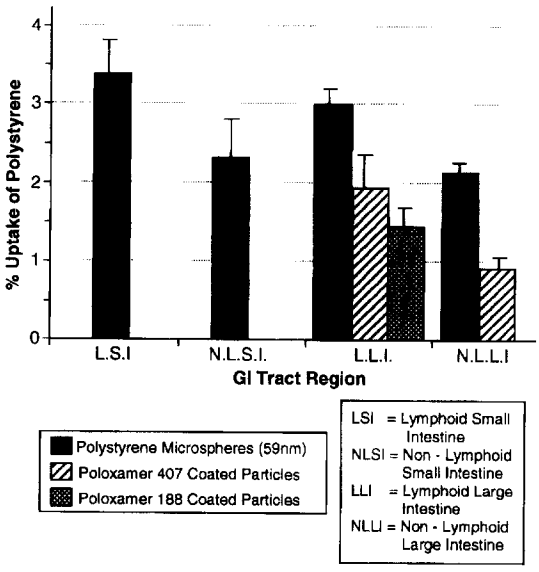

Fig. 2. Percentage uptake of poloxamer-coated and uncoated polystyrene microspheres (59 nm), in rat GI tract samples.

for the coated particles correlated with various studies on oral particulate uptake which have shown that hydrophobic particles are taken up to a greater extent than hydrophilic particles (LeFevre et al., 1985; Pappo and Ermak, 1989; Eldridge et al., 1990; Jepson et al., 1993a; Jepson et al., 1993b). Adsorbed poloxamer surfactants exhibit an inhibitory effect on polystyrene particle uptake by the RES after intravenous administration (Illum et al., 1986; Illum et al., 1987a), diverting the particles away from the liver and spleen to other tissue sites (Illum and Davis, 1983; Illum and Davis, 1984; Illum and Davis, 1987; Porter et al., 1992). The inhibitory effect of the poloxamers on RES uptake was attributed to dysopsonic (Van Oss et al., 1975; Illum et al., 1986; Lee et al., 1989) and anti-adhesive effects (Rutter and Vincent, 1980; Silberberg, 1983). These effects may also have been responsible for the inhibition of polystyrene uptake in the GI tract. Recently Durrer and co-workers (Durrer et al., 1994a; Durrer et al., 1994b) have measured the affinity of polystyrene latex to intestinal tissues, finding smaller particles (230 nm) adsorbing more rapidly than larger particles (670 nm) and that poloxamers 235, 237, 238 and 407 reduced the level of adsorption of latex in ex vivo experiments.

The mucoadhesive properties of the poloxamer surfactants may also have played a part in inhibiting particle uptake. Pimienta et al. (1992) reported that the mucoadhesion of poloxamer surfactants proceeds via an initial immediate adhesion of the POE chains to mucus through secondary forces, followed by their diffusion into mucus where they undergo molecular interactions. Although mucoadhesion was initially believed advantageous for oral drug delivery by a variety of mechanisms (Lehr et al., 1992), the advantages may be limited by the short turnover time (approximately 2 h) of

the intestinal mucus gel layer (Lehr et al., 1991). Thus a mucoadhesive system can be rapidly lost into the lumen on shedding of the mucus layer to which it is adhering, and a new mucus layer generated in its place.

In contrast to the findings in the small intestine for surfactant-coated particles, polystyrene uptake was detected in the large intestine (Fig. 2). Regional variations in the GI tract of the surface characteristics and uptake properties of the M cells may occur. Rabbit caecal M cells display differences in the uptake of antigenic material in comparison to jejunal M cells, and the caecal lymphoid patches of rabbits differ from other locations of GALT in their morphology, lectin-binding patterns and probably also function, suggesting that the numerous locations of GALT may have different functions and different antigen sampling methods, depending on the intestinal antigens and the microbial milieu (Gebert and Hach, 1993). Other regional variations in the GALT have been reported in sheep, dogs (HogenEsch et al., 1987; HogenEsch and Felsburg, 1990; HogenEsch and Felsburg, 1992), rabbits (Jepson et al., 1993c; Jepson et al., 1993d), cattle (Parsons et al., 1991) and mice (Clark et al., 1993; Clark et al., 1994).

An alternative explanation might be that the adsorbed surfactant layer may completely or partially desorb in vivo. Although the in vitro results demonstrate the stability of the coating over a 1-month period, and in vitro stability of poloxamer coatings has also been demonstrated by Wallis and Müller (1990) over a 7-day period, the situation in vivo is more complex. Lee et al. (1989) showed that certain types of poloxamer coatings on polystyrene particles were displaced by human serum albumin (HSA), fibrinogen and whole plasma. Illum et al. (1986), found that although poloxamer 188 caused an initial reduction in the liver uptake of polystyrene particles, the effect did not persist, which the authors attributed to a displacement of the adsorbed coating by plasma components with a stronger affinity for the particle surface.

Our results, however, show that it is possible to alter the uptake profile of polystyrene particles by the device of physically adsorbing poloxamer surfactant.

4. Conclusion

It was possible to manipulate the uptake profile of the polystyrene particles by modifying their surface properties with adsorbed poloxamer 188 and 407 surfactants. No polystyrene uptake of surfactant-coated particles was detected in the small intestine. Polystyrene uptake of coated particles was shunted to the large intestine, where only 3% of poloxamer 407 and 1.5% of poloxamer 188 coated particles were taken up, in contrast to 10% uptake across the entire GI tract of uncoated polystyrene particles.

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