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Surface modification of i.v. injectable biodegradable nanoparticles with poloxamer polymers and poloxamine 908

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Summary

Polyester nanoparticles (polylactide, polylactide/glycolide and poly(hydroxybutyrate)) for intravenous injection were produced by the solvent evaporation technique using poly(vinyl alcohol) as stabilizer. Modification of the particle surface was attempted by adsorption of hydrophilic compounds (coating) to avoid RES uptake in vivo. Adsorption of ethoxylated polymers (poloxamer polymers, Poloxamine 908) and ethoxylated surfactants (glycerol monooleate, nonyl phenols) was not possible or of low efficiency compared to polystyrene model carriers. The particle production technique was therefore modified by using different stabilizers in the solvent evaporation process. This resulted in coating layers up to 10 nm and distinctly increased surface hydrophilicity of the particles. However, the hydrophilicity of these coated particles was not regarded as sufficient to avoid opsonization and subsequent RES clearance.

Introduction

The major obstacle for drug delivery with intravenous colloidal carriers is the uptake of the particles by macrophages of the reticuloendothelial system (RES), mainly Kupffer cells of the liver (up to 90% of the injected dose within 5 min) and macrophages of the spleen (2–5%) (Müller, 1991). The uptake by liver and spleen could be reduced by the adsorption of ethoxylated block copolymers (poloxamer) on particles (Illum and Davis, 1983, 1984). The uptake was eliminated using model carriers (60 nm polystyrene particles) surface-modified by the adsorption of the ethoxylated block copolymer poloxamine 908. The adsorption (coating) layer protected the particles against uptake and they circulated in the blood (Davis et al., 1986; Illum et al., 1987). The task for the future will be transferring this success with non-biodegradable polystyrene particles to particles which are biodegradable (e.g., polyester) and can be applied clinically.

For this transfer to biodegradable particles it is essential to understand the mode of action of

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the coating layers. This allows controlled adjustment of the properties of biodegradable particles. Steric stabilization by adsorption layers larger than 10 nm is claimed as being the protective mechanism against macrophage uptake (Davis and Illum, 1988; Harper et al., 1991). However this is in contradiction to:

(1) The in vitro phagocytosis of particles with sterically stabilizing coats when the surface is immunologically recognized (Müller and Rudt, 1991) and

(2) The RES uptake of poloxamine 908 coated polyester particles despite the adsorption layer being greater than 10 nm (Koosha, 1989; Müller, 1991).

The protection of colloidal particles by adsorption layers against phagocytosis is attributed to the formation of an uncharged, extremely hydrophilic surface (Müller and Blunk, 1989) and the absence of complement activating groups (Müller, 1991). The hydrophilic surface avoids recognition because it reduces the adsorption of blood components (e.g., opsonins, apolipoproteins) (Blunk et al., 1991). Hydrophilicity alone cannot prevent RES uptake if complement activating groups, e.g., hydroxyl groups (Wegmüller et al., 1986), are present. Particles made hydrophilic by a coating layer of poly(vinyl alcohol) are cleared by the RES (Koosha, 1989; Müller, 1991) which is attributed to the presence of OH groups. The hydrophilicity of the poloxamer and poloxamine coated particles is a function of the coating layer thickness (Müller and Blunk, 1989; Müller, 1991). It increases with increasing thickness, being optimal for poloxamine 908 at 13–15 nm. To produce biodegradable carriers avoiding RES uptake it is therefore necessary to achieve coating layers distinctly larger than 10 nm. However, up to now coating of poly(lactic acid) (PLA) particles produced by solvent evaporation techniques has been carried out with very little success or has not been possible at all.

In this study we have tried to achieve a coating by modifying particle production using various stabilizers in the solvent evaporation process. The stabilizer changes the surface properties of the particles by incorporation into the surface (Koosha et al., 1989) and affects the subsequent adsorption layer of ethoxylated polymers. Alternatively, we examined other ethoxylated surfactants for coating purposes. The stability of the coating layers against desorption was determined and their potential to reduce RES uptake is discussed.

Materials and Methods

Materials

Poly(β-hydroxybutyrate) (PHB, Mol. Wt 21 000) was purchased from Malborough (Stockton-on-Tees, U.K.); poly(lactic acid) (PLA, inherent viscosity 0.3, Mol. Wt 28 000) and poly(lactic acid-co-glycolic acid) (50:50) (PLA/GA, inherent viscosity 0.4, Mol. Wt 34 000) were a gift from Boehringer Ingelheim (Germany). Poly(vinyl alcohol) (Mol. Wt 10 000) was purchased from Sigma (Deisenhofen, Germany), sodium dodecyl sulfate (SDS), tridecyl sulfate, tetradecyl sulfate and hexadecyl sulfate from Hoechst (Frankfurt/Main, Germany).

Ethoxylated blockcopolymers poloxamer 184, 188, 338 and 407 and poloxamine were provided by Erbslöh (Düsseldorf, Germany). GAF (Wayne, U.S.A.) provided ethoxylated nonyl phenols (Antarox CO 970 (50 mol EO), Antarox CO 990 (100 mol EO), Gafac RE 960 (50 mol EO with a phosphate group at the end of the EO chain)) and the dinonyl phenol Antarox DM 970 (150 mol EO).

Ethoxylated glycerols were synthesized by Goldschmidt (Essen, Germany): SH E075 contained 75.2 mol ethylene oxide and 24.76 mol propylene oxide (statistically distributed in the chains); hydroxyl number, 27; iodine number, 8.25; acid number, 0.85; SH EO100 contained 100 mol ethylene oxide; hydroxyl number, 28.1; iodine number, 8.8; acid number, 0.9; molecular weights, approx. 4 000.

Polystyrene particles (PS, 80 nm) were obtained from Polysciences (Warrington, U.S.A.), and fetal calf serum from Biochrom (Berlin, Germany).

The 5 ml diafiltration cell (model 3) was purchased from Amicon (Witten, Germany) and used with an Amicon Diaflo XM-300 membrane (Mol.

Wt cut-off, 300000, retaining capacity, 65% of IgG with Mol. Wt 160000).

Methods

The particles were produced by the solvent evaporation technique (Beck et al., 1980; Tice and Lewis, 1983; Koosha, 1989). The polymer was dissolved in methylene chloride (1%) and predispersed in an aqueous solution containing a stabilizer (0.5% PVA, 0.2% SDS or other alkyl sulfates or a saturated solution of hexadecyl sulfate) using an Ultra-turrax homogenizer (pre-mix) and then sonicated using a sonic probe (200 W, 5 min) (Labsonic, Braun-Diesel-Biotechnik, Melsungen, Germany). The methylene chloride was evaporated under reduced pressure (600 mbar at 20°C). The particle size was determined by photon correlation spectroscopy (PCS), yielding diameters of about 250 nm for particles produced with PVA (PHB-PVA, PLA-PVA and PLA/GA-PVA) and of 90 nm for particles produced with SDS (PHB-SDS, PLA-SDS and PLA/GA-SDS).

Coating of the particles was performed by mixing equal volumes of particle suspension (0.2%) and polymer/surfactant solution (0.2%) and incubation overnight.

For diafiltration, 4 ml of the suspension of coated particles (0.1% particles, 0.1% block copolymer or ethoxylated surfactant) were filled into the cell and diafiltered at a constant flow rate (0.12 ml/min) for 48 h as described previously (Wallis and Müller, 1990a).

PCS was used to measure the coating layers of the particles in water and the adsorbed layers in serum.

Results and Discussion

Coating of polystyrene and polyester nanoparticles

The ethoxylated blockcopolymers formed adsorption layers between 12 and 15 nm on 80 nm polystyrene nanoparticles (Table 1). They adsorb on the surface with their hydrophobic center part (polypropylene oxide), the hydrophilic chains protruding into the dispersion medium forming a new, hydrophilic surface (Cosgrove et al., 1981; Lee et al., 1989). The thickness of the poloxamer and poloxamine layers was in the range reported previously on 60 nm latex (Müller, 1991). The similar coating layers are a result of the comparable surface hydrophobicity of uncoated 60 and 80 nm polystyrene particles. Polystyrene particles larger in size which are less hydrophobic possess distinctly thinner adsorption layers. Poloxamine 908 forms layers of only 6.0–8.0 nm on particles of 145 nm–5.6 μm (Müller et al., 1992). This explained the extremely thin or undetectable layers on biodegradable particles produced with PVA as stabilizer, e.g., PHB-PVA particles (Table 1). The PVA is attached to the surface of the polyester particles reducing its hydrophobicity. PHB particles produced with PVA were found to be as hydrophilic as polystyrene particles after coating with poloxamine 908 (Koosha, 1989; Müller, 1991). They are therefore too hydrophilic to adsorb a distinct amount of surfactants.

TABLE 1

Coating layer thickness of ethoxylated polymers and surfactants on polystyrene particles (PS) and on polyester particles produced with PVA as stabilizer in the solvent evaporation process (n.d. = not detectable)

Coating material	Coating layer thickness (nm)			
	PS	PHB	PLA	PLA/GA
Poloxamine 908	15.1	2.5	n.d.	n.d.
Poloxamer 338	14.7	3.3	2.9	n.d.
Poloxamer 407	12.5	n.d.	n.d.	1.5
Antarox CO 990	7.5	2.2	1.0	n.d
Antarox CO 970	6.0	n.d.	n.d.	n.d.
Gafac RE 960	2.8	n.d.	n.d.	n.d.
SH EO 100	6.3	n.d.	n.d.	n.d.
SH EO 75	5.1	n.d.	n.d.	n.d.

We hoped to improve the adsorption by using ethoxylated surfactants possessing a more hydrophobic anchor part. However, replacing the polypropylene oxide by glycerol monooleate (SH EO75, SH EO100) or by a nonyl phenol did not lead to thicker coating layers (Table 1). The increased hydrophobicity of the anchor groups did not increase the hydrophobic interaction between solute and particle to an extent sufficient to enhance adsorption.

TABLE 2

Coating layer thickness of ethoxylated polymers and surfactants on polystyrene particles (PS) and on polyester particles produced with SDS as stabilizer in the solvent evaporation process

Coating material	Coating layer thickness (nm)			
	PS	PHB	PLA	PLA/GA
Poloxamine 908	15.1	9.3	5.5	8.1
Poloxamer 338	14.7	9.0	5.5	6.5
Poloxamer 407	12.5	8.1	4.1	5.5
Antarox CO 990	7.5	6.2	4.3	5.0
Antarox CO 970	6.0	4.5	2.7	3.4
Gafac RE 960	2.8	2.2	2.2	3.0
SH EO 100	6.3	3.5	1.9	2.8
SH ED 75	5.1	3.0	2.4	3.1

Producing biodegradable particles with SDS led to the incorporation of the stabilizer into the particle surface as could be shown by static secondary ion mass spectrometry (SSIMS) (Koosha et al., 1989). The PHB-SDS particles were distinctly more hydrophobic than the PHB-PVA particles but less hydrophobic than polystyrene particles as determined by hydrophobic interaction chromatography (Koosha, 1989; Müller, 1991). The adsorption layers were therefore distinctly thicker on polyester particles produced with SDS than on those produced with PVA. However, they were below the layer thickness observed on polystyrene particles (Table 2). Controlled modification of the surface of biodegradable nanoparticles can therefore be used to facilitate a coating.

In general, the coating layers observed on PLA-SDS and PLA/GA-SDS nanoparticles were thinner than those on PHB-SDS (Table 2). This cannot be explained on the basis of differences in the surface hydrophobicities of the polymers themselves. They are similar as indicated by the contact angles of approx. 68° (Galazka, 1987). The differences were therefore attributed to differences in the amount of SDS incorporated in the particle surface.

Particles were produced using stabilizers with increasing length of hydrocarbon chain (C12–C16) to possibly increase the nanoparticle surface hydrophobicity and consequently enhance the coating. The length of the hydrocarbon chain had litttle effect on the coating layers of poloxamine 908 (Table 3). However, a distinct increase from 3.2 to 7.9 nm was observed for the ethoxylated dinonyl phenol (Antarox DM 970, Table 3). This increase for Antarox DM 970 might be explained by the enhanced interaction between the nonyl groups of the surfactant and the hydrocarbon chains on the surface. The hydrocarbon chains are at least partially located on the surface as can be concluded from SSIMS spectra. Alternatively, enhanced incorporation of stabilizers with longer hydrocarbon chains might occur into the particle surface. The small effect on poloxamine adsorption can be explained by differences in the driving forces of the adsorption. The affinity to the surface plays an important role for Antarox whereas the adsorption of the block polymers is mainly entropy driven (Carstensen et al., 1990; Wesemeyer et al., 1992).

Stability of coating layers against desorption

The coating layers of poloxamer polymers and poloxamine 908 on polystyrene particles are extremely stable against desorption. The adsorption

TABLE 3

Coating layer thickness of poloxamine 908 and Antarox DM 970 on poly(hydroxybutyrate) (PHB) and polylactide/glycolide (PLA/GA) particles produced with different alkyl sulfates as stabilizer in the solvent evaporation process

Stabilizer in solvent evaporation process	Thickness of coating layer (nm)		
	Poloxamine 908 on PLA/GA	Poloxamine 908 on PHB	Antarox DM 970 on PLA/GA
Dodecyl sulfate	7.2	9.3	3.2
Tridecyl sulfate	–	–	3.4
Tetradecyl sulfate	–	–	4.4
Hexadecyl sulfate	8.9	10.8	7.9

TABLE 4

Coating layer thickness on polystyrene particles and on polylactide / glycolide particles produced with SDS (PLA / GA-SDS) before diafiltration (t = 0 h) and after 48 h of diafiltration (t = 48 h)

Coating material	Thickness of coating layer (nm)			
	On polystyrene		On PLA/GA-SDS	
	$t = 0$ h	$t = 48$ h	$t = 0$ h	$t = 48$ h
Poloxamine 908	13.3	12.0	7.8	9.3
Poloxamer 338	15.3	13.7	8.0	7.2
Poloxamer 407	11.2	11.3	6.7	7.7
Poloxamer 188	6.1	8.6	3.1	4.0
Poloxamer 184	3.7	5.0	1.5	3.5
Antarox DM 970	8.1	6.5	–	–

process is very rapid whereas desorption proceeds very slowly (Wallis and Müller, 1990a). The reduction in coating layer thickness after 48 h of diafiltration was minor (< 1.6 nm) for surfactants adsorbed on polystyrene particles (Table 4). The stability of the block copolymers can be explained by their multipoint attachment (Silberberg, 1990). However, even Antarox DM 970 proved very stable on polystyrene particles. The coating layers of ethoxylated surfactants were thinner but of similar stability against desorption (Table 4). This is important for the intravenous injection of coated biodegradable nanoparticles for site-specific drug delivery. The adsorbed polymer layer should not be desorbed or displaced in the blood.

No displacement occurs when injecting Poloxamine 908 coated 60 nm polystyrene particles. They were reported to circulate in the blood for hours (Davis et al., 1986; Illum et al., 1987). From the diafiltration experiments a similar stability in the blood is therefore expected for Poloxamine 908 coating on biodegradable particles. Adsorbed polymer layers can remain on surfaces even in surroundings which contain competing compounds. The layers behave as irreversibly bound (Silberberg, 1990). Blood components are therefore unlikely to cause displacement. However, to escape RES recognition the poloxamine coat needs to be not only of identical stability but simultaneously of identical hydrophilicity as on polystyrene particles.

Assessment of the potential of coated polyester particles to avoid RES uptake

It was possible to obtain a polymer coat when displacing PVA by other stabilizers in the particle production process. Poloxamine 908 proved to yield the thickest layers of all coating materials investigated. However, the poloxamine 908 layers on the various polyester particles possessed a maximum thickness of 10 nm. The hydrophilicity of the block copolymer coating layers (and consequently the dysopsonic effect) was found to increase with coating layer thickness (Müller and Blunk, 1989). Only coating layers with a thickness of about 13–15 nm were sufficiently hydrophilic to avoid opsonisation in the blood and subsequent uptake by the reticuloendothelial system (Illum et al., 1987). The coating layers achieved were distinctly below this value. The interaction of coated particles with serum proteins can be determined by measuring the ζ-potential in serum (Blunk and Müller, 1989). Low ζ-potentials of about -5 mV were obtained in the case of slight interaction with serum compounds (e.g., poloxamine 908 coated polystyrene particles), with higher ζ-potentials (of about -15 mV) being determined for opsonized particles taken up by the RES (e.g., insufficiently coated or uncoated polystyrene particles). The thickest coating layers of poloxamine 908 on PHB-SDS particles obtained with previous production batches (approx. 10–12 nm) possessed a ζ-potential of -14 mV (Koosha, 1989; Müller, 1991) indicating strong interaction with serum proteins. These particles were rapidly taken up by liver and spleen after intravenous injection in New Zealand White rabbits (Koosha, 1989; Müller, 1991). The strong interaction of polyester particles with serum could be confirmed by PCS measurements. PCS was previously employed to determine the dysopsonic effect of coating materials by direct determination of the thickness of adsorbed protein layers on particles in serum (Wallis and Müller, 1990b). Adsorbed layers on PLA/GA particles coated with polymers creating thin layers increased distinctly in thickness after serum incubation due to the adsorption of large amounts of protein (Table 5). The increase was less pronounced for poloxamine 908 and poloxamer 407 (up to 3.7 nm,

TABLE 5

Coating layer of polymers on particles in water, and thickness of adsorbed layers on uncoated particles (consisting of serum proteins) and on coated particles (adsorbed polymer and serum proteins) in serum

Carrier material	Coating material	Coating layer in water	Adsorbed layer in serum
PLA/GA	uncoated	–	7.2
	poloxamine 908	7.8	11.5
	poloxamer 338	8.0	10.4
	poloxamer 407	6.7	8.5
	poloxamer 188	3.1	8.9
	poloxamer 184	1.5	8.6
Polystyrene	uncoated	–	33.1
	poloxamine 908	13.3	12.6
	poloxamer 407	11.2	9.1

Table 5). In contrast, no significant increase was detectable for poloxamine 908 and poloxamer 407 coated polystyrene particles avoiding liver/spleen uptake (Table 5). Modification of the particle production techniques therefore did not lead to coating layers sufficiently thick and hydrophilic to minimize interaction with serum compounds. None of the surface-modified particles produced to date is likely to avoid RES uptake.

Conclusions

Adsorption of ethoxylated coating polymers and ethoxylated surfactants on biodegradable polyester particles could be achieved by modification of the production technique. PVA was displaced by anionic stabilizers with hydrocarbon chain lengths from C12 to C16. The use of ethoxylated surfactants led to thin layers although they possessed a more hydrophobic anchor part than the polymers. The coating layers of the block copolymers were about 10 nm thick but not sufficiently hydrophilic to minimize interaction with plasma proteins similar to poloxamine 908 coated 60 nm polystyrene particles. The modification of the polyester surface, however, is a promising approach to remove the major obstacle for i.v. drug delivery with biodegradable polyester nanoparticles, the insufficient coating.

References

Beck, L.R., Pope, V.Z., Cowsar, D.R., Lewis, D.H. and Tice, T.R., Evaluation of a new three-month injectable contraceptive microsphere system in primates (baboons). *Contracept. Deliv. Syst.*, 1 (1980) 79–86.

Blunk, T., Hochstrasser, D.F., Rudt, S. and Müller, R.H., Two-dimensional electrophoresis in the concept of differential opsonization – an approach to drug targeting. *Arch. Pharm.*, 324 (1991) 706.

Blunk, T. and Müller, R.H., Uptake of charged serum components as a selection criterion for i.v. drug carriers avoiding liver/spleen uptake. *Arch. Pharm.*, 322 (1989) 755.

Carstensen, H., Müller, B.W. and Müller, R.H., Microcalorimetric studies of the adsorption of ethoxylated surfactants on polystyrene particles. *Arch. Pharm.*, 323 (1990) 776.

Carstensen, H., Müller, B.W. and Müller, R.H., Adsorption of ethoxylated surfactants in nanoparticles. I. Characterization by hydrophobic interaction chromatography. *Int. J. Pharm.*, 67 (1991) 29–37.

Cosgrove, T., Croweley, T.L. and Vincent, B., The configuration of adsorbed polymers at the solid-solution interface. *Faraday Symp. Chem. Soc.*, 16 (1981) 101–108.

Davis, S.S., Douglas, S., Illum, L., Jones, P.D.E., Mak, E. and Müller, R.H., Targeting of colloidal carriers and the role of surface properties. In Gregoriadis, G., Senior, J. and Poste, G. (Eds), *Targeting of Drugs with Synthetic Systems*, Plenum, New York, 1986, pp. 123–146.

Davis, S.S. and Illum, L., Polymeric microspheres as drug carriers. *Biomaterials*, 9 (1988) 111–115.

Galazka, V., A study of the surface modification of polymers when treated by oxygen plasma and irradiation. Sandwich Training Report. Pharmacy Department at Nottingham University, 1987.

Harper, G.R., Davies, M.C., Davis, S.S., Tadros, T.F., Irving, M.P. and Waters, J.A., Steric stabilisation of microspheres with grafted polyethylene oxide reduces phagocytosis by rat Kupffer cells in vitro. *Biomaterials*, 12 (1991) 695–700.

Illum, L. and Davis, S.S., Effect of the non-ionic surfactant Poloxamer 338 on the fate and deposition of polystyrene microspheres following intravenous administration. *J. Pharm. Sci.*, 72 (1983) 1086–1089.

Illum, L. and Davis, S.S., The organ uptake of intravenously administered colloidal particles can be altered using a non-ionic surfactant (Poloxamer 338). *FEBS Lett.*, 167 (1984) 79–82.

Illum, L., Davis, S.S., Müller, R.H., Mak, E. and West, P., The organ distribution and circulation time of intravenously injected colloidal carriers sterically stabilized with a block copolymer – Poloxamine 908. *Life Sci.*, 40 (1987) 367–374.

Koosha, F., Preparation and characterisation of biodegradable polymeric drug carriers. Ph.D. Thesis, University of Nottingham, 1989.

Koosha, F., Müller, R.H., Davis, S.S. and Davies, M.C., The surface chemical structure of poly(β-hydroxybutyrate) microparticles produced by solvent evaporation process. *J. Controlled Release*, 9 (1989) 149–157.

Lee, J., Martic, P.A. and Tan, J.S., Protein adsorption on Pluronic copolymer-coated polystyrene particles. *J. Coll. Interf. Sci.*, 131 (1989) 252–266.

Müller, R.H., *Colloidal Carriers for Controlled Drug Delivery and Targeting*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1991.

Müller, R.H. and Blunk, T., Properties of poloxamer and poloxamine coating layers on different sized polystyrene model drug carriers. *Arch. Pharm.*, 322 (1989) 699.

Müller, R.H., Heinemann, S., Blunk, T. and Ruth, S., PCS and zeta potential characterization of model drug carriers – essential information for the interpretation of in vitro and in vivo data. In Satelle, D.B. and Harding, S. (Eds), *Laser Light Scattering in Biochemistry*, 1992, pp. 425–440.

Müller, R.H. and Rudt, S., Optimization of a chemiluminescence based cell culture assay to study the affinity of surface-modified drug carriers to macrophages. *Proc. 18th Int. Symp. Controlled Release Bioact. Mater.*, (1991) 219–220.

Silberberg, A., Macromolecules at surfaces. In Gurny, R. and Junginger, H.E. (Eds), *Bioadhesion - Possibilities and Future Trends*. Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1990, pp. 16–28.

Tice, T.R. and Lewis, D.H., Microencapsulation process. US *Patent 4,389,330*, June 21 (1983).

Wallis, K.H. and Müller, R.H., Stability of poloxamer and poloxamine coating films on polystyrene nanoparticles. *Acta Pharm. Technol.*, 36 (1990a) 127–132.

Wallis, K.H. and Müller, R.H., Surface-modified colloidal drug carriers – determination of the dysopsonic effect by PCS. *Acta Pharm. Technol.*, 36 (1990b) S34.

Wegmüller, E., Mondandon, A., Nydegger, U.E. and Descoeudres, C., Biocompatibility of different hemodialysis membranes: activation of complement and leukopenia. *Int. J. Artif. Organs*, 9 (1986) 85–92.

Wesemeyer, H., Müller, B.W. and Müller, R.H., Adsorption of ethoxylated surfactants on nanoparticles. II. Determination of adsorption enthalpy by microcalorimetry. *Int. J. Pharm.*, 89 (1993) 33–40.