Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmaceutics and Biopharmaceutics 60 (2005) 361–372
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Protein adsorption patterns on poloxamer- and poloxamine-stabilized solid lipid nanoparticles (SLN)

Torsten M. Göppert, Rainer H. Müller*

Department of Pharmaceutical Technology, Biotechnology and Quality Management, The Free University of Berlin, Institute of Pharmacy, Berlin, Germany

Received 21 October 2004; accepted in revised form 7 February 2005
Available online 6 April 2005

Abstract

Solid lipid nanoparticles (SLN) were produced using a full range of poloxamer polymers and poloxamine 908 for stabilization. The protein adsorption pattern acquired on the surface of these particles after intravenous injection is the key factor determining the organ distribution. Two-dimensional polyacrylamide gel electrophoresis (2-DE) was employed for determination of particle interactions with human plasma proteins. The objective of this study was to investigate changes in the plasma protein adsorption patterns in the course of variation of the polymers stabilizing the SLN. Considerable differences in the protein adsorption with regard to preferential adsorbed proteins were detected for the different stabilizers. Possible correlations between the polyethylene oxide (PEO) chain length and the adsorption of various proteins (first of all apolipoproteins) are shown and discussed. Besides the study of protein adsorption patterns, the total protein mass adsorbed to the SLN was also evaluated using the bicinchoninic acid (BCA)-protein assay. The knowledge concerning the interactions of proteins and nanoparticles can be used for a rational development of particulate drug carriers. Based on the findings presented in this paper, we anticipate that the in vivo well-tolerable SLN are a promising site-specific drug delivery system for intravenous injection.

Keywords: Solid lipid nanoparticles; Poloxamer; Poloxamine; Protein adsorption patterns; Two-dimensional polyacrylamide gel electrophoresis; Site-specific drug delivery

1. Introduction

Intravenously (i.v.) injected nanoparticulate drug carriers are one attempt to realize the 'magic bullet' concept postulated by Ehrlich, which means to target drugs specifically to their site of action [1]. To allow a controlled development of site-specific carriers, it is a prerequisite to know the factor determining the organ distribution of the carriers. Since more than half a century, intensive studies are being undertaken to identify this organ distribution determining factor [2–4]. Meanwhile, it is generally accepted that the protein adsorption pattern, acquired after intravenous injection of the particles, is the crucial factor determining the organ distribution [5,6]. Two-dimensional polyacrylamide gel electrophoresis (2-DE) has proven to be a powerful tool to determine these plasma protein adsorption patterns of nanoparticulate carriers [6]. In case opsonins such as immunoglobulin or complement factors are adsorbed, the particles are immediately cleared by the macrophages of the mononuclear phagocytic system (MPS) [7]. In case these opsonins are missing, the particles show a reduced or no uptake by MPS cells [8]. Such particles can circulate in the blood stream, e.g. poloxamine 908-coated 60 nm polystyrene model carriers [3,9]. An even more pronounced stealth effect could be achieved if the so-called dysopsonins (albumin or apolipoproteins) were preferentially adsorbed on the particles surface. In case of a pronounced albumin, adsorption occurs on the particle surface, the surface renders more hydrophilic reducing in general the adsorption of other blood proteins. To target a specific drug to a site other than the MPS, firstly the drug-loaded particles need to escape from the MPS recognition, and secondly, they need to have preferentially adsorbed on their surface a protein, which is able to mediate the uptake to the target cells. According to this

* Corresponding author. Department of Pharmaceutics, Biopharmaceutics and Quality Management, The Free University of Berlin, Institute of Pharmacy, Kelchstr. 31, 12169 Berlin, Germany. Tel.: +49 30 838 50 696; fax: +49 30 838 50 616.
E-mail address: mpharma@zedat.fu-berlin.de (R.H. Müller).

0939-6411/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2005.02.006

theory, targeting to the brain was achieved using Tween 80-stabilized polymeric nanoparticles [10–12]. Apolipoprotein E (apoE) could be identified as the component mediating the brain targeting [13].

Most of the organ distribution, and related protein adsorption studies, were performed either with non-biodegradable model particles (e.g. polystyrene) or using polymeric nanoparticles. This latter system usually exhibits the problems such as too slow in vivo biodegradation and release of toxicologically problematic degradation products such as formaldehyde (e.g. polyalkylcyanoacrylate nanoparticles [14]). Therefore, in this study, solid lipid nanoparticles (SLN) were used. The SLN showed a very good tolerability in vitro cell culture studies [15] but also after intravenous bolus injection of up to 1 g lipid/kg [16,17]. Based on this, the SLN have the potential to be accepted by the regulatory authorities and being used in patients.

Efficient surface modifiers that can be used to reduce the MPS uptake are polyethylene oxide (PEO)-containing nonionic block co-polymers, i.e. poloxamers and poloxamines, also known as Pluronic® and Tetronic®, respectively. It was shown in vivo that particles coated with such polymers could circulate longer in the blood (poloxamine 908) [3] or be accumulated in the bone morrow (poloxamer 407) [18]. Moreover, studies on bovine brain endothelial cells have demonstrated that poloxamer 235 (Pluronic P85) single chains ('unimers') inhibited the glycoprotein P (P-gp) efflux pump, thus enhancing drug accumulation in these cells [19]. In contrast, poloxamer 235 micelles induced transient drug accumulation in the same cells. The efflux is directed to the same side where the micelles were administered. However, the direction of transport can be modified by conjugating the micelles with a ligand capable of adsorptive endocytosis in the cell [20], such as apoE.

Therefore, the SLN investigated in this study were surface-modified with a range of these interesting polymers to assess whether they show adsorption patterns known from model or polymeric particles leading to site-specific accumulation (targeting). In case such patterns are found, SLN could be used as a well-tolerated i.v. targeting delivery system.

2. Materials and methods

2.1. Materials

Cetyl palmitate was obtained from Henkel KG (Düsseldorf, Germany), poloxamer 184, 235, and 407 from C.H. Erbslöh (Düsseldorf, Germany), poloxamer 188 from BASF (Ludwigshafen, Germany) and poloxamer 237, 238, 338, and poloxamine 908 from ICI Surfactants (Middlesborough, UK). Immobiline DryStrips (pH 3–10, nonlinear) were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden) and human plasma was obtained from the German Red Cross (Berlin, Germany) and stored at −70 °C. For 2-DE, all chemicals were of analytical grade. Acrylamide was purchased from Serva (Heidelberg, Germany) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), ammonium persulfate and piperazine diacrylamide (PDA) from BioRad (Munich, Germany). All other chemicals according to [21] were obtained either from Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland). The BCA regent-kit for protein quantification was obtained from Pierce (Rockford, USA).

2.2. Methods

2.2.1. Solid lipid nanoparticles: preparation and physicochemical characterization

SLN were produced by the hot homogenization method as described previously [22,23] using cetyl palmitate as matrix lipid (10.0% (w/w)). The polymer concentration (poloxamer, poloxamine) was kept constant at 1.2% (w/w). The lipid was melted at approximately 5 °C above its melting point and dispersed by an Ultra-Turrax T 25 (Janke and Kunkel, Staufen, Germany) in a hot surfactant mixture heated at the same temperature. The obtained pre-emulsion was then homogenized at the same temperature using a Micron LAB 40 (APV Systems, Unna, Germany), applying three homogenization circles at 500 bar. A hot nanoemulsion resulted; cooling led to crystallization of the lipid and formation of the SLN.

The mean diameter of the nanoparticle population was assessed by photon correlation spectroscopy (PCS) using a Malvern Zetasizer IV (Malvern instruments, Malvern, UK). PCS gives the mean diameter of the particle population and the polydispersity index (PI) ranging from 0 (monodisperse) to 0.50 (very broad distribution). The content of micrometer particles was determined by laser diffractometry (LD) using a Coulter LS 230 (Beckman-Coulter, Krefeld, Germany). LD yields a volume distribution. The diameters 50, 90, 95 and 99% were chosen as parameters characterizing the size distribution (i.e. a diameter of 95% means that 95% of the particle volume is below the given size).

Zeta potential measurements were performed using the Malvern Zetasizer IV. The field strength applied was 20 V/cm. The SLN were dispersed in distilled water having a conductivity adjusted to 50 μS/cm by addition of NaCl-solution, pH was about 5.8. The conversion of the electrophoretic mobility to the zeta potential was done using the Helmholtz–Smoluchowsky equation.

Water contact angle on a cetyl palmitate film was measured using a Krüss G1 contact angle goniometer (Krüss, Hamburg, Germany) equipped with a sessile dropper. Care was taken to perform measurements on part of the film on which there were no cracks. Ten readings were taken and the mean value was obtained.

2.2.2. Sample preparation

To analyze the protein adsorption on the SLN, suspensions of the particles containing constant surface areas