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Supercooled smectic nanoparticles: Influence of the matrix composition and in vitro cytotoxicity

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ABSTRACT

Cholesteryl nonanoate (CN), myristate (CM), palmitate (CP) and oleate (CO) alone or in combination were evaluated as matrix lipids for the preparation of supercooled smectic nanoparticles with a high stability against recrystallization during storage.

The phase behavior of the cholesterol esters in the bulk was studied by polarizing light microscopy, differential scanning calorimetry (DSC) and small angle X-ray scattering (SAXS). Colloidal dispersions with pure and mixed cholesterol ester matrices were prepared by high-pressure melt homogenization and characterized by photon correlation spectroscopy, laser diffraction combined with polarizing intensity differential scattering, DSC and SAXS. The morphology of selected formulations was studied by freeze-fracture electron microscopy. All smectic nanoparticles with a mixed cholesterol ester matrix were stable against recrystallization when stored at room temperature. Nanoparticles with a pure CN and mixed CM/CN matrix with a high fraction of CN (60% of the whole lipid matrix) could even be stored at 4 °C for at least 18 months without any recrystallization.

As smectic nanoparticles are studied especially with regard to parenteral administration of lipophilic drugs, the cytotoxicity of selected formulations was compared with that of a clinically used colloidal fat emulsion (Lipofundin MCT) in the murine fibroblast cell line L929 using the sulforhodamine B assay. The supercooled smectic nanoparticle formulations display a good overall cell compatibility although in some cases their cytotoxicity was slightly higher than that of Lipofundin MCT.

1. Introduction

Lipoproteins are endogenous transport vehicles in the blood for physiological lipids and lipophilic substances. They are also carriers for therapeutic agents, e.g., hydrophobic porphyrins (Mazière et al., 1991). Lipoproteins consist of a lipid core (triglycerides, and different ratios of cholesterol and cholesterol esters depending on the type of lipoproteins) surrounded by a phospholipid monolayer and attached apolipoproteins (Scanu and Wisdom, 1972). The apolipoproteins on the surface determine the binding to specific lipoprotein receptors. Colloidal fat emulsions and LDL-like formulations are therefore being investigated as carriers for poorly water soluble, lipophilic drugs (Tamilvanan, 2004; Dias et al., 2007). Colloidal fat emulsions are being clinically used over decades for parenteral nutrition (Carpentier and Dupont, 2000) and several drug loaded colloidal fat emulsions are commercially available, e.g., with diazepam (Diazepam-Lipuro®, Stesolid®), etomidate (Etomidate-Lipuro®) and propofol (Diprivan®, Propofol-MCT Fresenius®). Since drug release from lipid emulsion droplets is often very fast under sink conditions for drugs with moderate lipophilicity (Magenheim et al., 1993; Washington and Evans, 1995; Takino et al., 1994, 1995; Hosokawa et al., 2003), solid lipid nanoparticles were introduced in the 1990s (Mehnert and Mäder, 2001). It was expected that the crystalline lipid matrix would result in a restricted mobility of incorporated drugs and in a sustained release. Rapid release of at least a fraction of the incorporated drugs is, however, often observed also in solid lipid nanoparticle dispersions (Zur Mühlen et al., 1998; Rosenblatt and Bunjes, 2006). Furthermore, the highly ordered crystalline state usually limits the drug loading capacity of the matrix lipid (Westesen et al., 1997). The liquid crystalline state with a high structural order but mobility at the molecular level may offer a compromise between the isotropic liquid and highly ordered crystalline state. Supercooled smectic nanoparticles prepared from physiological cholesterol esters

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were therefore introduced as a novel lipid-based colloidal delivery system for poorly water soluble drugs (Kuntsche et al., 2004). The smectic state of the nanoparticles may offer advantages in terms of physicochemical stability and drug loading capacity over other types of lipid nanoparticles like fat emulsions and solid lipid nanoparticles.

Cholesteryl myristate (CM), which has a completely reversible thermal phase behavior, was chosen as model cholesterol ester for the evaluation of this new type of carrier (Kuntsche et al., 2004, 2005; Kuntsche and Bunjes, 2007). The basis for the development of smectic cholesterol ester nanoparticles is the strong supercooling effect of the smectic phase in the colloidal state. Although bulk CM is a solid lipid at room temperature, the nanoparticles retain the smectic state over considerable time when stored at room temperature (Kuntsche et al., 2004). In previous studies, the physicochemical properties and the influence of different parameters such as particle size and stabilizer system on the recrystallization tendency of CM nanoparticles were studied in some detail (Kuntsche et al., 2004, 2005). Model drugs like ibuprofen, etomidate and miconazole could be incorporated into the colloidal dispersions in a comparably high concentration of 10% related to the matrix lipid. The association of the drugs with the matrix lipid was clearly detectable by alterations of the thermal behavior of the nanoparticles (Kuntsche et al., 2004). Ideally, in the interest of chemical stability, a robust formulation should allow long-term storage at 4 °C, which is not the case for smectic CM nanoparticles crystallizing above or around 0 °C (Kuntsche et al., 2005). For this reason, supercooled smectic nanoparticle matrix compositions that are potentially more stable with respect to nanoparticle recrystallization during storage were investigated here. Cholesterol esters with low recrystallization tendency – cholesteryl nonanoate (CN) and oleate (CO) – were used as matrix lipids alone and in mixture with long-chain saturated cholesterol esters – cholesteryl myristate (CM) and palmitate (CP). To evaluate the physiological compatibility which is a prerequisite for a drug delivery system particularly for parenteral administration, the in vitro cytotoxicity of selected formulations was compared to that of a commercially available fat emulsion for parenteral nutrition (Lipofundin MCT).

2. Material and methods

2.1. Materials

Cholesteryl myristate (CM, Sigma, ICN, purity >99%), cholesteryl nonanoate (CN, Acros Organics, purity >99%), cholesteryl palmitate (CP, Acros Organics, purity >95%), cholesteryl oleate (CO, Acros Organics, purity >95%), purified soybean phospholipid Lipoid S100 (S100, Lipoid KG, D-Ludwigshafen), sodium glycocholate (SGC, Sigma), poloxamer 188 (POL, Lutrol F68, BASF, D-Ludwigshafen), thiomersal (Caesar & Loretz, D-Hilden), glycerol (Solvay, D-Rheinberg) and water for injection Ph.Eur. (prepared by successive filtration, deionization, reverse osmosis and distillation using an in-house water purification system) were used for the preparation of the dispersions.

The frozen stock culture of murine fibroblasts (L929) was obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-Braunschweig, DSMZ cell line ACC2). The RPMI 1640 medium (with HEPES, L-glutamine and phenol red) was from PAA Laboratories (A-Pasching), fetal calf serum (FCS), trypsin solution (500 U/ml), penicillin/streptomycin solution (10,000 IU/ml penicillin and 10 mg/ml streptomycin), trypan blue solution 0.4% and TRIS base from Sigma. Sulforhodamine B (SRB) was from Fluka (CH-Buchs) and trichloroacetic acid from Merck (D-Darmstadt). Lipofundin MCT 10% was a kind gift of B. Braun Melsungen GmbH (D-Melsungen). Phosphate buffered saline pH 7.4 (136.89 mM NaCl, 2.68 mM KCl, 1.47 mM KH_2PO_4 and 8.20 mM $Na_2HPO_4 \cdot 2H_2O$, all from Merck, D-Darmstadt) used in cell culture was sterilized by autoclaving (Varioklav 25T, Thermo Scientific).

2.2. Composition and preparation of the dispersions

Dispersions containing 5 or 10% cholesterol ester(s) as matrix lipid(s) and 4 or 8% stabilizer(s) (concentrations are w/w, see Tables 1 and 2) were prepared by high-pressure melt homogenization as described earlier (Kuntsche et al., 2004). In brief, the lipids were molten at about 70 °C (CO) or 90–95 °C (CM, CN, CP and lipid mixtures) and the aqueous phase containing the stabilizer(s) heated to the same temperature was added to the lipid melt. The crude emulsions prepared by Ultra-Turrax vortexing (1 min, 25,000 rpm, Ultra-Turrax T8, Ika Labortechnik, D-Staufen) at the same temperature were transferred to the pre-heated high-pressure homogenizer (Microfluidizer M110S, Microfluidics, USA-Newton) and homogenized continuously for 5 min with 850–1100 bar at high temperatures (Tables 1 and 2). The hot emulsions were filtered (pore size 0.2 or 5 μm) to remove potential contaminations from the homogenization process, cooled to room temperature and stored at 23 °C. A fraction of each dispersion was stored at 4 °C (except for the dispersion prepared for cell culture experiments). Unless otherwise stated, the results described below refer to the nanoparticles stored at 23 °C.

The aqueous phase of the dispersions contained 2.25% glycerol for isotonization and 0.01% thiomersal for preservation (both w/w). Thiomersal was used to avoid any microbiological growth during storage and handling of the dispersions during the physicochemical studies (Table 1). Non-preserved dispersions were prepared for the cell culture experiments (Table 2). These dispersions were autoclaved under standard conditions (Kuntsche and Bunjes, 2007) using an industrial automated autoclave (Fedegari Autoclavi, I-Albuzzano). The size (PCS), osmolality (Knauer Semi-Micro Osmometer, Knauer, D-Berlin) and pH (MP 225 with a InLab 415 electrode, Mettler Toledo, D-Giessen) was measured after autoclaving the dispersions.

2.3. Determination of particle size and size distribution

Particle sizes and size distributions of the dispersions were determined by photon correlation spectroscopy (PCS, Zeta Plus Particle Sizer, Brookhaven Instruments) and laser diffraction combined with PIDS technology (LD-PIDS, Coulter LS 230 Particle Sizer, Beckman-Coulter) as described earlier (Kuntsche et al., 2004). The values given are averages of 6–8 measurements over 5 min (PCS, measured at 90° and 25 °C in purified and filtered water) or 90 s per run (LD-PIDS, applying the Mie theory and assuming refractive indices of 1.33 and 1.45 for the dispersant and the particles, respectively). The results of PCS measurements are given as *z*-average and polydispersity index (PDI) calculated by cumulant analysis. For the LD-PIDS measurements, the arithmetic mean, median (D50), mode (maximum of the distribution) and the D99 (size at 99% of the cumulative size distribution) obtained from the volume weighted size distribution are given.

2.4. Differential scanning calorimetry (DSC)

DSC measurements were made on a Pyris 1 (PerkinElmer) or on a Micro-DSC III calorimeter (Setaram). In the first case, approximately 1–5 mg of the bulk lipids or 8–15 mg of the dispersions were accurately weighed into standard aluminum pans. An empty pan was used as reference. The bulk lipids were measured with different scan rates (1, 5 and 10 °C/min) and the colloidal dispersions