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Research paper

Drug distribution in nanostructured lipid particles

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Propylene glycol monocaprylate (Capryol® 90) (PubChem CID: 53630264)
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ABSTRACT

The targeted design of nanoparticles for efficient drug loading and defined release profiles is even after 25 years of research on lipid-based nanoparticles still no routine procedure. It requires detailed knowledge about the interaction of the drug with the lipid compounds and about its localisation and distribution in the nanoparticle. We present here an investigation on nano-sized lipid particles (NLP) composed of Gelucire and Witepsol as solid lipids, and Capryol as liquid lipid, loaded with Dexamethasone, a glucocorticoid used in topical treatment of inflammatory dermal diseases. The interactions of Dexamethasone, which was spin-labelled by 3-(Carboxy)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (DxPCA), with its microenvironment are monitored by EPR spectroscopy at 94 GHz at low temperatures. The mobility of the spin-labelled drug was probed by X-band EPR at room temperature. In order to relate the magnetic and dynamic parameters deduced from EPR to the local environment of the spin probe in the NLP, investigations of DxPCA in the individual lipid compounds were carried out. The magnetic parameters reflecting the polarity of DxPCA's environment as well as the parameters describing the mobility of the drug reveal that in the case of colloidal dispersions of the lipids and also the NLP DxPCA is attached to the surface of the nanoparticles. Although the lipophilic drug is almost exclusively associated with the NLP in aqueous solution, dilution experiments show, that it can be easily released from the nanoparticle.

1. Introduction

Colloidal systems like suspensions, emulsions and micellar or vesicular solutions are in common use for the topical application of pharmaceuticals with limited water solubility [1]. As an alternative to oil/water emulsions and liposomes, lipid-based nanoparticles have been introduced as drug delivery systems for poorly water-soluble drugs [2,3]. These nanoparticles have potential advantages over liposomes and emulsions: They show room/body temperature stability, no organic solvents are needed throughout preparation, a broad application spectrum, and the controlled release of the incorporated drug is possible [4,5]. Additionally the composition of nanoparticles from different lipids and surfactants allows for tuning to different drug solubilities [4,6,7].

Lipid-based nanoparticles can be classified into solid lipid nanoparticles (SLN) or heterogeneous, presumably liquid/solid phase matrix particles, called nanostructured lipid carriers (NLC). NLC were developed to increase the loading capacity compared to SLN [4]. A number of investigations concerning the size and shape of SLN and NLC have been published [8,9]. Either a crystalline or a supercooled melt matrix core is found, depending on the compositions [8–10].

Investigations concerning the drug distribution within the nanoparticles are rare [10,11]. One report made use of model compounds like the fluorescent nile red and found a localisation in the liquid lipid phase of NLC [12]. Diverging models assume the inclusion of substrates either in the core, the outer shell, as a colloidal

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dispersion in the lipid matrix or in a homogeneous distribution [13,14]. It is generally accepted that lipid-based nanoparticles including NLCs improve the penetration of drugs through the skin [5,15,16].

For the treatment of inflammatory diseases and in particular dermal diseases Dexamethasone (Dx) is regularly used [17]. It is a synthetic glucocorticoid with anti-inflammatory and immunosuppressive effects [17]. Due to its low solubility in water (89 mg/l at 25 °C) co-solvation or usage of liquid lipids is necessary.

Electron paramagnetic resonance (EPR) is a well-established method for probing the local environment of a spin probe with respect to polarity, proticity and mobility [18,19]. NO-based spin labels have been used as probes in membrane proteins [20,21], lipids [18,19] and liposomes [22,23]. EPR can furthermore provide information on the loading of molecules onto, localisation in and release from nano-sized carrier systems [11,24–26].

In the present study, multi-frequency EPR was used to determine the microenvironment of the spin-labelled Dx within lipid-based carrier systems to elucidate the localisation of the drug within the carrier. Control measurements were performed on different sizes of the NLP, on its individual compounds and mixtures at room temperature and under cryogenic conditions (80 K). Furthermore, dilution experiments were performed to study the release kinetics and particle stability.

2. Materials and methods

2.1. Preparation of spin-labelled Dexamethasone (DxPCA)

DxPCA was synthesised by refluxing Dx with PCA, 4-(Dimethyl)-aminopyridin and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide in dichloromethane for 3 h with subsequent column-chromatographic purification [27].

2.2. Synthesis of nano-sized lipid particles (NLP)

The lipid components Gelucire® 50/13 (stearoyl macrogolglycerides, GATTEFOSSÉ GmbH, Bad Krozingen, Germany), Witepsol® S55 (solid triglycerides containing hydrogenated coco-glycerides, beeswax and ceteareth-25, CREMER OLEO GmbH & Co. KG, Hamburg, Germany), and Capryol® 90 (propylene glycol monocaprylate, Gattefossé GmbH, Bad Krozingen, Germany) were mixed in a ratio of 5:3:1 (w/w) and melted at 60 °C together with 0.5% (w/w) PCA-labelled Dx. The 10% (w/w) lipid nanosuspension was prepared from a nanoemulsion by pouring ultrapure water of the same temperature to the lipid melt and high-shear-homogenisation for 5 min. Finally, the nanoemulsion was cooled to room temperature to solidify the lipid phase and thus, to obtain the lipid nanosuspension. The lipid nanosuspension was filtered through 0.7 µm glass fiber filters (Whatman® GF/F; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to eliminate precipitated drug crystals from the water phase.

Two different sizes of DxPCA loaded NLP were produced to study the effect of loading. Furthermore, DxPCA was incorporated into single lipid components of the NLP and in selected lipid combinations, with and without dispersion in water to identify the microenvironment of DxPCA in the NLP.

2.3. Particle size determination

The mean particle size of the NLP was determined by a Zetasizer® Nano ZS (Malvern Instruments GmbH, Herrenberg, Germany) equipped with a He-Ne laser (633 nm) at a backscattering angle of 173 ° and cell temperature of 25 °C. The lipid nanosuspension was diluted to a ratio of 1:10 (v/v) with ultrapure water. 10 runs were performed, from which the mean particle size and polydispersity index with standard deviation were calculated. The particle size of the DxPCA NLP is 134 ± 3 and 242 ± 18 nm, respectively.

To investigate the release behaviour of DxPCA, dilution series were performed: a NLP solution (NLP diameter: 134 nm ± 3 nm) loaded with DxPCA was diluted to ratios of 1:10 and 1:50 with water, followed by measurements with a Zetasizer® Nano ZS.

2.4. Electron paramagnetic resonance (EPR) spectroscopy

Cryogenic temperature (80 K) EPR measurements were performed using a W-band (94 GHz) EPR spectrometer (Bruker Elexsys E680 S/W-Band) equipped with a Teraflex EN600-1021H probe head (Bruker Biospin, Karlsruhe, Germany). Room temperature measurements at X-band (9 GHz) were performed on a laboratory-built spectrometer employing a SHQ probe head (Bruker Biospin, Karlsruhe, Germany). The temperature was monitored by an Oxford ITC 503 temperature controller. The magnetic field was calibrated using a N@C_{60} standard [28].

For all cryogenic measurements the samples were prepared in quartz tubes of 0.87/0.7 mm (outer diameter (o.d.)/inner diameter (i.d.) for W-band (VitroCom Inc., Mountain Lakes, USA). Room temperature EPR at X-band was performed with 2 mm/1 mm (o.d./i.d.) capillaries (QSIL GmbH, Langewiesen, Germany). The experimental parameters are different for each microwave band and are shown in the respective figure legend.

The evaluation of the magnetic parameters (g-matrix, ^{14}N hyperfine couplings (*hfc's*), rotational correlation time and line width) for determining the environment polarity and mobility of DxPCA was performed using the EasySpin toolbox [29], a Matlab package (The MathWorks GmbH, Ismaning, Germany) for EPR spectra simulation.

3. Results

3.1. Localisation of DxPCA within the NLP

DxPCA was investigated in NLP and in different lipid solutions of the NLP components by X-band (9.4 GHz) and W-band (94 GHz) at room temperature and at 80 K to evaluate the microenvironment properties polarity and viscosity, and the mobility of the drug in the used carrier system.

The g-matrix principal value g_{xx} and the ^{14}N *hfc* parameter A_{zz} are sensitive to the polarity and proticity of the microenvironment of a nitroxide compound [30,31]. To study the *g*-matrix and ^{14}N *hfc*, DxPCA loaded to NLP was investigated by high frequency (W-band) EPR spectroscopy at 80 K (Fig. 1). Fig. 1 shows the low temperature spectra of DxPCA in aqueous dispersions of Gelucire/Witepsol, in NLP (242 nm), in the liquid lipid Capryol, and of DxPCA dissolved in water. Additionally, EPR spectra of DxPCA in Gelucire, Witepsol, Gelucire/Witepsol mixture and Gelucire/water dispersion were recorded and are shown in Fig. S1. Water was chosen as a reference, since it acts as the solvent surrounding the NLP and has one of the highest polarity and proticity properties [32]. The g-matrix and the ^{14}N *hfc* matrix A of DxPCA in the different mixtures/solvents were evaluated by simulation of the experimental spectra and are given in Table 1. Thereof, the principal components g_{xx} and A_{zz} are most sensitive to the solvent properties polarity and proticity are indicated in Fig.1.

The g_{xx}- and A_{zz}-values of DxPCA in water are typical for a spin label in an aqueous environment [31]. All g_{xx}-values for DxPCA in the lipids are upshifted and all A_{zz} are downshifted compared to