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Influence of nanocarrier type and size on skin delivery of hydrophilic agents

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ABSTRACT

The nanoparticulate carrier systems solid lipid nanoparticles (SLN) and dendritic core-multishell (CMS) nanotransporters gained interest for the topical treatment of skin diseases as they facilitate the skin penetration of loaded lipophilic drugs. Here, we studied if these carrier systems are also suitable drug delivery systems for more hydrophilic agents using the dye rhodamin B as model compound. Furthermore, the influence of the particle size on the skin penetration was investigated. Loading rhodamin B onto SLN (250–340 nm) and CMS nanotransporters (20–30 nm), the dye amount increased significantly in viable epidermis and dermis as compared to a conventional cream. CMS nanotransporters were most efficient. Creating nanoparticles of 50–200 nm demonstrated only marginal size effect for the skin penetration. Therefore, the superiority of the CMS nanotransporters seems to be attributed to the character of the nanoparticles and not to its smaller size.

1. Introduction

Solid lipid nanoparticles (SLN) and a new typed dendritic core-multishell (CMS) nanotransporters can improve the topical therapy of skin diseases by enhancing dermal drug penetration (Küchler et al., 2009; Schäfer-Korting et al., 2007) and – possibly – limiting side effects (for review see Manjunath et al., 2005). Due to the hydrophobic matrix of SLN, rather lipophilic drugs are considered for loading, whereas hydrophilic agents are expected to be poorly loaded onto SLN. In contrast, depending on the specific structure, synthetic polymers and CMS nanotransporters can encapsulate lipophilic as well as hydrophilic agents and transport them to polar as well as nonpolar environments (Alvarez-Roman et al., 2004; Luengo et al., 2006; Radowski et al., 2007). In a previous study with the lipophilic dye nile red ($\log P = 5$), CMS nanotransporters were superior to SLN for the enhancement of skin penetration (Küchler et al., 2009). A size dependent effect was assumed as SLN (150–170 nm) are considerably larger than the CMS nanotransporters (20–30 nm).

This study aimed to investigate and compare the efficacy of SLN, CMS nanotransporters and a standardized cream for the dermal penetration of hydrophilic compounds loading the water soluble model dye rhodamin B ($\log P < 1$) (Merck, 2008). In fact, with hydrophilic agents in general skin penetration is rather poor (Potts and Guy, 1992). Aiming to unravel also the influence of the particle size on the skin penetration, lipid nanocapsules (LNC, 50–200 nm) were prepared, too, since neither SLN nor CMS nanotransporters can be generated at defined sizes covering the range of interest. LNC are, similar the SLN, lipid nanocarriers but composed of a neutral oil phase and soybean lecithin. The dispersion is stabilized in water by a non-ionic surfactant (Lamprecht et al., 2002).

2. Materials and methods

2.1. Materials

Compritol® 888 ATO (glyceryl behenate) and neutral oil were a gift from Gattefossé (Weil a. Rh., Germany). Soybean lecithin (Lipoid® S75) and polyethylene glycol-660 hydroxystearate (PEG-HS, Solutol® HS15) were gifts from Lipoid (Ludwigshafen, Germany). The emulsifier Lutrol F68® (Poloxamer 188) was obtained from BASF (Ludwigshafen, Germany). Oil-in-water cream (as described by the "Deutscher Arzneimittelcodex 2004") was supplied by Caelo (Hilden, Germany). Rhodamin B was obtained from Sigma–Aldrich (Munich, Germany). Pig skin of the axillary region from donor animals (breed: "Deutsche Landrasse, 45–55 kg, 8–12 weeks old) was provided by the Department of Comparative Medicine and Facilities of Experimental Animal Sciences, Charité (Berlin, Germany) and was processed as described (Schäfer-Korting et al., 2008).

Abbreviations: CMS, dendritic core-multishell; LD, laser diffraction; LNC, lipid nanocapsules; PCS, photon correlation spectroscopy; PEE, penetration enhancing effect; PI, polydispersity index; SLN, solid lipid nanoparticles.

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2.2. Test preparations and dye loading

All the test preparations contained the dye (rhodamin B or nile red) at a concentration of 0.004%. Rhodamin B – loaded SLN, composed of 10% solid lipid (Compritol® 888 ATO) and 2.5% emulsifier, were prepared close to nile red loaded particles (Lombardi Borgia et al., 2005). Due to its hydrophilic properties, however, rhodamin B was dissolved in the water and the Poloxamer was added. The solution was added to the melted lipid. For rhodamin B encapsulation, CMS nanotransporters (20–30 nm; 5 g/l), synthesized at the Department of Organic Chemistry, Freie Universität Berlin (Berlin, Germany), were dispersed in water first followed by dissolving the dye. The solution was stirred for 12 h and subsequently filtrated via size exclusion chromatography to separate loaded CMS nanotransporters from free dye molecules. The final rhodamin B concentration was obtained by diluting the sample with pure water.

For the LNC preparation, nile red was dissolved in methylene chloride and added to the neutral oil phase. Thereafter, LNC formulations at nominal sizes of 50 nm, 100 nm, and 200 nm were prepared by using a phase inversion processing (Lamprecht et al., 2002). The formulations were filtrated for enhanced microbiologic stability.

Dye loaded cream served as reference in the skin penetration studies with rhodamin B. The according amount of the dye was incorporated into base cream homogeneously.

2.3. Particle characterization

Photon correlation spectroscopy (PCS, Malvern Zetasizer ZS, Malvern Instruments, Malvern, UK) was used to evaluate the mean particle size (*z*-average) and the polydispersity index (PI) as a degree for the width of the distribution. Laser diffraction (LD, Coulter LS 230, Miami, FL) was studied to detect larger particles with micrometer size. The LNC were analyzed for their particle size and size distribution using a Zeta Plus (Brookhaven Instruments, Holtsville, NY) at an angle of 90°.

To determine the loading capacity of SLN, the sample was diluted 1:10 with water and filtrated with a 0.2 μm filter (Sartorius, Göttingen, Germany). The fluorescence intensity of the filtrate was quantified using a microplate reader (FLUOstar Optima, BMG Labtech, Offenburg, Germany), setting the excitation wavelength to 530 nm and emission wavelength to 570 nm.

2.4. Skin penetration

To characterize the skin penetration validated test procedures for the Franz cell set up in the finite-dose approach (Schäfer-Korting et al., 2008) and picture analysis (Lombardi Borgia et al., 2005) were performed. Pig skin was used as it is comparable to human skin and already well established for in vitro testing (Diembeck et al., 1999; Schäfer-Korting et al., 2008). 35 μl of the test preparations loaded with rhodamin B 0.004% or nile red 0.004% were applied to pig skin mounted onto Franz cells. CMS nanotransporters, SLN and cream were tested in parallel using skin of the same donor animal. The experiments were repeated with the skin of another two donor pigs. The LNC dispersions were studied in three independent experiments, too.

After 6 h exposure, the skin was removed, cleaned of remaining formulation and frozen at −80 °C. Vertical slices of 20 μm thickness were subjected to normal and fluorescence light microscopy (20× magnifications, BZ-8000, Keyence, Neu-Isenburg). The fluorescence was recovered in the red band exciting the samples at 560 nm and setting the camera integration time to 1/45 s for rhodamin B or 1/4 s for nile red data evaluation. The arbitrary pixel brightness (ABU) values were evaluated using image analysis software BZ Analyser (Keyence, Neu-Isenburg, Germany) and the relative dye content within the stratum corneum, viable epidermis and dermis was quantified, respectively (Lombardi Borgia et al., 2005).

2.5. Statistics

The presented data (arithmetic mean values ± standard error of the mean) resulted from three independent experiments. Statistical analysis is based on the Wilcoxon matched pairs test; $p \leq 0.05$ is considered to indicate a difference. By relating mean ABU values obtained from rhodamin B uptake following CMS nanotransporters and SLN application to the uptake data from dye loaded cream the penetration enhancing effect (PEE) was calculated. The PEE of the cream is 1 by definition.

3. Results

For SLN, PCS measurements revealed an average size of 250–340 nm with a polydispersity index (PI) ≤ 0.250, higher concentrations of rhodamin B were related to the increase in size. The production of samples with rhodamin B concentrations ≥ 0.008% was restricted by the immediate gelation of the SLN dispersions. The determined loading capacity was 99%. The average size of the CMS nanotransporters was 20–30 nm. The mean particle size of LNC dispersions was 60 nm, 90 nm, and 185 nm. LNC had a low PI (0.04–0.1) and showed a monomodal particle size distribution, respectively.

The cutaneous uptake of rhodamin B loaded onto SLN, CMS nanotransporters and a conventional cream was studied, respectively. Representative fluorescence staining (Fig. 1A–C) indicates an enhanced dye penetration into the viable epidermis and dermis after application of the nanoparticulate carrier systems as compared to the cream. Data evaluation by picture analysis demonstrated the superiority of the nanoparticles (Fig. 1D). As the here applied experimental settings do not allow for a quantitative analysis of absolute dye concentration, data evaluation was performed by calculating the penetration enhancing effect (PEE) over cream. CMS nanotransporters were most efficient, respectively. The penetration of rhodamin B into the viable epidermis increased 8.8-fold following the application of SLN and 11.5-fold following the application of CMS nanotransporters. The penetration enhancing effects of the nanoparticles loaded with rhodamin B for the stratum corneum were with SLN PEE = 1.09 and with CMS nanotransporters PEE = 1.22.

To determine the influence of the particle size the skin penetration of nile red loaded lipid nanocapsules (60 nm, 90 nm, and 185 nm) was tested, too. No statistical significant differences were observed (Fig. 2).

4. Discussion

To improve the notoriously low skin penetration of drugs in topical dermatotherapy and transdermal therapy nanoparticular carrier systems are of increasing interest (Schäfer-Korting et al., 2007). By the means of the lipophilic dye nile red the efficacy of various lipid-based nanoparticles and CMS nanotransporters was compared (Küchler et al., 2009; Lombardi Borgia et al., 2005). The new-typed dendritic CMS nanotransporters were most promising (Küchler et al., 2009). Moreover, nile red penetration into the skin was also studied using polymer nanoparticles (Alvarez-Roman et al., 2004).

For completion of the first results, here we investigated whether SLN and CMS nanotransporters may be also suitable for penetration enhancement of hydrophilic agents as compared to conventional cream. Due to the lipid matrix of SLN a low entrapment efficacy for hydrophilic drugs was assumed. In contrast, CMS nanotransporters encapsulate hydrophilic agents effectively due to their polar polyethylene glycol core (Radowski et al., 2007). In our studies, the

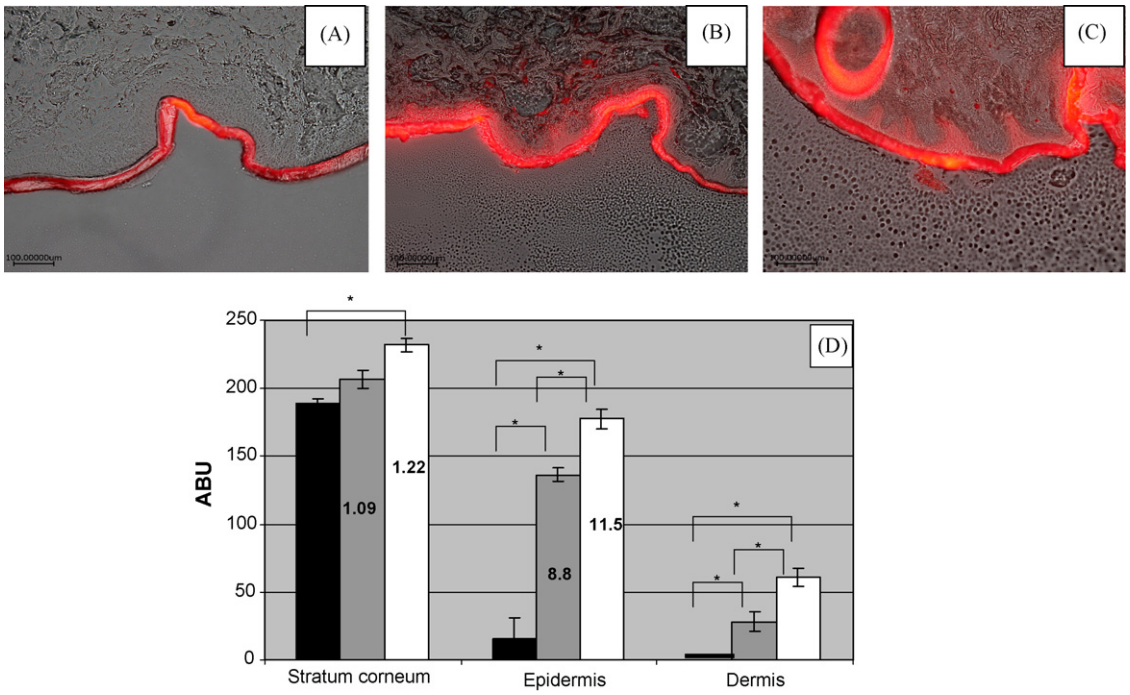

Fig. 1. Rhodamin B penetration into pig skin: staining of pig skin following the application of 0.004% rhodamin B loaded cream (A), SLN (B) and CMS nanotransporters (C) for 6 h. The representative pictures taken from the identical donor animal are obtained by superposing normal light and fluorescence images of the same area. (D) The arbitrary pixel brightness values (ABU) obtained by fluorescence picture analysis (cream, black columns; SLN, grey columns; CMS nanotransporters, white columns, $n = 3$). The inserted numbers give the respective enhancement of penetration over cream, *differences ($p \leq 0.05$).

water soluble rhodamin B served as hydrophilic model dye. For further characterization of SLN loaded with hydrophilic agents we prepared nanoparticles with different dye concentrations. Those SLN were about 250–340 nm, dependent on the dye concentration. The particle size of SLN which were used for the penetration studies (0.004% rhodamin B) was 250 nm. In contrast, nile red loaded SLN were about 150–170 nm (Küchler et al., 2009; Lombardi Borgia et al., 2005). The dependence of the particle size on the concentration of the loaded agent was also observed with another model agent: CAT-1, a hydrophilic spin probe (data not shown). Here, the particle size was 250–420 nm, gelation occurred at concentrations > 0.05%. Therefore, we assume the localization of hydrophilic substances within the poloxamer layer of the SLN close to the aqueous phase, since higher dye concentrations enable stronger intermolecular interactions, e.g. hydrogen bonds. Thus, gelation is facilitated and the limiting concentration when gelation occurs is well explained. The latter may depend on the molecular size of the loaded substance as the limiting concentration of rhodamin B (479 g/mol) is lower than of CAT-1 (209 g/mol). Importantly, the increase of the particle sizes also points to an efficient loading of the dye. This is confirmed by a measured loading capacity of 99%.

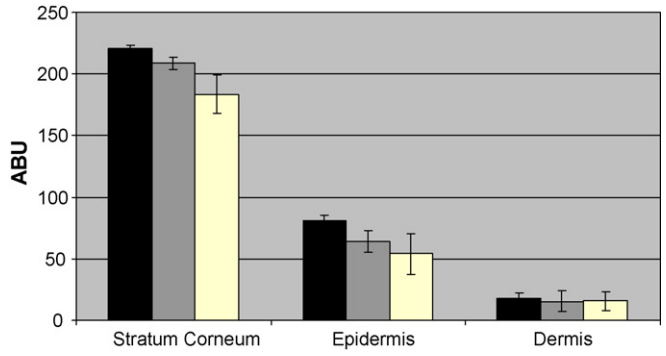

Fig. 2. Effects of nanoparticle size on dye penetration into pig skin: arbitrary pixel brightness values (ABU) following the application of lipid nanocapsules 60 nm (black columns), 90 nm (gray columns), 185 nm (white columns) in diameter loaded with 0.004% nile red ($n = 3$).

The size of the CMS nanotransporters, however, does not depend on the physicochemical properties of the guest molecule (Radowski et al., 2007). Particle sizes of the rhodamin B loaded particles were in the same range (20–30 nm) as with nile red loaded CMS nanotransporters (Küchler et al., 2009).

Next, we compared the cutaneous uptake of rhodamin B loaded onto SLN, CMS nanotransporters and a conventional cream, respectively. Once more CMS nanotransporters were most efficient, respectively. Following the application of SLN and CMS nanotransporters the penetration of rhodamin B into the viable epidermis increased 8.8-fold with SLN and 11.5-fold with CMS nanotransporters. Thus, the findings of previous studies with the lipophilic dye nile red were confirmed (Küchler et al., 2009; Lombardi Borgia et al., 2005). In contrast, however, a relevant penetration enhancing effect of the nanoparticles loaded with rhodamin B did not exist for the stratum corneum (PEE < 1.3; Fig. 1D) whereas PEE values of nanoparticles loaded with nile red were 3.8 (SLN) and 7.8 (CMS), respectively (Küchler et al., 2009). Obviously, SLN and CMS nanotransporters are suitable carrier to facilitate the skin penetration of lipophilic and hydrophilic agents. The difference in the penetration profile of hydrophilic substances (low amount of dye in the stratum corneum) is due to the very lipophilic character of the stratum corneum that does not allow the accumulation of hydrophilic agents such as rhodamin B. Previous studies showed that the efficacy of the nanoparticulate carrier systems is not due to their lower lipid content and, thus, their lower viscosity compared to the cream (Lombardi Borgia et al., 2005). Additionally, poloxamer used for SLN stabilization was proven not to influence the skin penetration by altering its barrier properties (Blaschke et al., submitted for publication). Furthermore, in contrast to SLN and CMS nanotransporters the cream contains the penetration enhancer propylene glycol (10%) and was less efficient anyway. The PEE for the dermis

was not calculated as the measured ABU values were close to the limit of quantification derived from the assay validation.

The reasons for the superiority of the penetration enhancement by CMS nanotransporters over SLN are still ambiguous, a size effect appears most plausible. Therefore, we next investigated the influence of the particle size on the skin penetration. As to date it is not possible to prepare defined SLN or CMS nanotransporters in the size range of interest, the skin penetration of a model dye loaded to lipid nanocapsules (60 nm, 90 nm, and 185 nm) was determined. By the means of lipid nanocapsules we succeeded in the formation of dye loaded nanoparticles of defined size classes – yet by modifying the nanoparticle type.

According to previous experiences, we opted for the use of nile red once more. The major decline in nile red concentration from stratum corneum to epidermis and dermis following the application of LNC is well in accordance with nile red distribution following cream, SLN and CMS nanotransporters (Küchler et al., 2009). Unexpectedly, the influence of the particle size on the dye penetration into the skin is marginal and not significant ($p > 0.05$; Fig. 2) in the size range (60–185 nm) studied here including the size of nile red – loaded SLN (170 nm) (Lombardi Borgia et al., 2005; Küchler et al., 2009) and close to the sizes of CMS nanotransporters. Previous studies proved that the penetration enhancing effect of SLN obviously results from intensive interaction between the skin lipids and the lipids of the nanoparticles (Küchler et al., 2009). The exact drug delivery mechanism of CMS nanotransporters still has to be elucidated, but these results indicate that the character and not the size of the particles determine preferentially the dermal penetration of drugs. Nevertheless, this assumption has to be confirmed with SLN and CMS nanotransporters once the preparation of those particles with defined overlapping sizes becomes possible.

In conclusion, SLN and CMS nanotransporters should be considered as drug delivery systems also for the topical application of hydrophilic substances. The nanoparticle size appears of subsidiary relevance for the enhancement of the skin penetration.

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