Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 317 (2006) 82–89
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Oral bioavailability of cyclosporine: Solid lipid nanoparticles (SLN®) versus drug nanocrystals

R.H. Müller [a], S. Runge [a], V. Ravelli [b], W. Mehnert [a], A.F. Thünemann [c], E.B. Souto [a,*]

[a] *Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin, Kelchstr. 31, D-12169 Berlin, Germany*
[b] *Institute of Neurosurgery, University of Pisa, Pisa, Italy*
[c] *Division of Structure Analysis, Federal Institute for Materials Research and Testing, Richard Willstätter Strasse 11, 12489 Berlin, Germany*

Received 12 January 2006; received in revised form 23 February 2006; accepted 24 February 2006
Available online 31 March 2006

Abstract

For the development of an optimized oral formulation for cyclosporine A, 2% of this drug has been formulated in solid lipid nanoparticles (SLN™, mean size 157 nm) and as nanocrystals (mean size 962 nm). The encapsulation rate of SLN was found to be 96.1%. Nanocrystals are composed of 100% of drug. For the assessment of the pharmacokinetic parameters the developed formulations have been administered via oral route to three young pigs. Comparison studies with a commercial Sandimmun Neoral/Optoral® used as reference have been performed. The blood profiles observed after oral administration of the commercial microemulsion Sandimmun® revealed a fast absorption of drug leading to the observation of a plasma peak above 1000 ng/ml within the first 2 h. For drug nanocrystals most of the blood concentrations were in the range between 30 and 70 ng/ml over a period of 14 h. These values were very low, showing huge differences between the measuring time points and between the tested animals. On the contrary, administration of cyclosporine-loaded SLN led to a mean plasma profile with almost similarly low variations in comparison to the reference microemulsion, however with no initial blood peak as observed with the Sandimmun Neoral/Optoral®. Comparing the area under the curves (AUC) obtained with the tested animals it could be stated that the SLN™ formulation avoids side effects by lacking blood concentrations higher than 1000 ng/ml. In this study it has been proved that using SLN™ as a drug carrier for oral administration of cyclosporine A a low variation in bioavailability of the drug and simultaneously avoiding the plasma peak typical of the first Sandimmun® formulation can be achieved.

Keywords: Solid lipid nanoparticles; SLN; Drug nanocrystals; Sandimmun Neoral/Optoral®; Oral administration

1. Introduction

It is well known that the majority of the new chemical entities (approximately 60% of drugs) coming directly from synthesis are poorly soluble (Fichera et al., 2004). Consequently, many of these substances have bioavailability problems after oral administration. Injection or infusion as intravenous aqueous solution is in most cases not possible because the low solubility in water would require a too large administration volume. The use of solvent mixtures is often excluded as well, because more and more drugs are poorly soluble in aqueous and simultaneously in organic media. To make these new drugs available to the patients, there is a definite need for smart formulations to enhance bioavailability after oral administration or to solubilize the drugs and make them intravenously injectable. Of course, the first choice is the oral administration route.

Frequent approaches to enhance solubility and subsequently oral absorption are the use of cyclodextrins (Sridevi et al., 2003; Fernandes et al., 2003) and oral microemulsions, such as cyclosporine A (CycA)-loaded microemulsions used in the commercial product Sandimmun Neoral/Optoral® (Vonderscher and Meinzer, 1994; Meinzer et al., 1998). Limitations of these approaches are: (i) the size of drug molecules that need to fit into the cyclodextrin rings, and (ii) the ability to form a microemulsion after drug dissolution in the microemulsion components. Furthermore, the microemulsions are usually prepared having three to four components and, according to the phase diagrams for these formulations, their physicochemical stability is dependent on the temperature. Thus, to obtain a suitable microemulsion formulation its thermodynamic stability both at storage and

* Corresponding author. Tel.: +49 30 83850678; fax: +49 30 83850616.
E-mail address: mpharma@zedat.fu-berlin.de (E.B. Souto).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.02.045

at body temperature should be guaranteed. In addition, the use of microemulsions changes the pharmacokinetic profile of the absorbed drug. A nice example is CycA. The first commercial product containing CycA was Sandimmun® capsules consisting in an oily solution of drug (25–100 mg CycA dissolved in corn oil, 12.80% ethanol, emulsifier: macrogolglycerol-tri(oleate, linolate)). One disadvantage of this formulation was the variation in drug bioavailability ranging from 10 to 60% (Beveridge et al., 1981). The aim of re-formulating CycA was to avoid this variation in bioavailability, which was achieved when preparing a microemulsion. The variation of C_{max} and t_{max} values were distinctly reduced (Meinzer et al., 1998). However, the microemulsion exhibits a pronounced initial plasma peak above 1000 ng/ml being highly responsible for potential nephrotoxicity (Penkler et al., 1999; Runge, 1998).

An ideal formulation should show a similar low variation in bioavailability as the CycA microemulsion and simultaneously avoid the plasma peak as the first Sandimmun® formulation does. The aim of this study was to achieve such a blood profile by using solid lipid nanoparticles (SLN®) as a drug carrier for oral administration.

SLN were derived from o/w emulsions by replacing the liquid lipid (oil) by a solid lipid, i.e. a lipid being solid at room and simultaneously at body temperature (Müller et al., 1995; Müller et al., 2000; Müller and Souto, 2006). Due to their solid matrix, drug release from SLN can be modulated (zur Mühlen et al., 1998; Souto, 2005), which could be exploited to optimize the blood profile. The distinct advantage of SLN is that they fulfil the pre-requisites to market a product. The excipients used are of regulatorely recognized status, i.e. all lipids and surfactants used for oral dosage forms such as tablets, capsules and pellets can be employed. The excipients are of low costs and large-scale production is possible by using high pressure homogenization lines already approved for pharmaceutical industry, for example, for the production of parenteral emulsions such as Intralipid®. At the turn of the millennium a second generation of lipid nanoparticles was developed. This so-called nanostructured lipid carriers (NLC®) are prepared not from a solid lipid but from a blend of a solid lipid with an oil (Müller et al., 2000, 1998a; Souto, 2005).

Another approach to formulate poorly soluble drugs for oral administration is the development of drug nanocrystals (Müller et al., 2001). This alternative can be used for drugs for which the dissolution velocity in water is an absorption limiting step. It is well known that micronization of a drug powder increases its dissolution velocity (Rasenack et al., 2003). In fact, drug nanocrystals go down in size one dimension further, i.e. by a nanonization process. Another interesting feature is the increased saturation solubility of nanonized drugs compared to micronized or larger sized powders (Müller and Keck, 2004). Both increased surface area and increased saturation solubility enhance the dissolution velocity. Note that the decrease of particle size increases the curvature of the particles and thus the dissolution pressure, leading to an increase of the saturation solubility around the particles. For some drugs, the drug nanocrystal principle proved to be highly effective. For example, the oral bioavailability of danazol could be improved from 5.1 to 82.3% when replacing the microsuspension by nanosuspension (Liversidge, 2003).

In comparison to the above-mentioned SLN, drug nanocrystals have the advantage of being easier to produce. Microsuspensions can be transferred to nanosuspensions simply by pearl milling (Müller et al., 1998b; Irngartinger et al., 2004) or by high pressure homogenization (Müller et al., 1998b; Müller, 2001; Moschwitzer et al., 2004; Moschwitzer and Müller, 2006). On the other hand, SLN are composed of a lipid matrix, and lipids are known to promote absorption of some drugs (Charman, 2000). Therefore, another purpose of the present investigation was to compare the efficiency of SLN versus drug nanocrystals to enhance oral drug absorption using CycA as a model drug with practical relevance for potential market products.

2. Materials and methods

2.1. Materials

Cyclosporine A (purity ≥ 95%) was obtained form Pharmatec (Milan, Italy). The lipid Imwitor®900 (glycerol monostearate 40–50%) was provided by Cognis (Düsseldorf, Germany). Tagat®S and sodium cholate were purchased from Sigma (Deisenhofen, Germany). Water was obtained from a MilliQ System Millipore (Schwalbach, Germany). Sandimmun Neoral/Optoral® was obtained from a German chemist shop in Berlin.

2.2. Preparation of the tested formulations

The SLN were produced by dissolving CycA (2.0%, m/m) in the melted Imwitor®900 (8.0%) at approximately 5–10 °C above its melting point (56–61 °C). The CycA-containing melt was dispersed in water containing 2.5% (m/m) Tagat®S and 0.5% (m/m) sodium cholate as surfactants. The temperature of both phases was accurately controlled, being identical. Dispersion was performed by high speed stirring (8000 rpm, 1 min) using an Ultra-Turrax (IKA, Staufen, Germany). The obtained pre-emulsion was homogenized using a Micron LAB 40 (APV Homogenizers, Unna, Germany) applying 500 bar and three homogenization cycles.

The drug nanosuspension was prepared by dispersing the CycA powder (2.0%) in a surfactant solution of identical composition at room temperature (22–25 °C). Homogenization was performed using the Micron LAB 40. Due to the hardness of the crystalline material the suspension was homogenized at 1500 bar applying 20 homogenization cycles. These production conditions have been established by pre-formulation studies.

2.3. Particle size analysis

Particle size analysis was performed by photon correlation spectroscopy (PCS, $n = 10$) using a Zetasizer IV (Malvern Instruments, Malvern, United Kingdom). PCS yields a mean diameter of the bulk population (z-Ave, average) and the polydispersity index (PI) as measure of the width of the size distribution. For detection of larger sized particles, i.e. outside the measuring range of PCS (>3 μm), laser diffractometry (LD, $n = 3$) was

employed using a Coulter LS 230 (Beckmann-Coulter, Krefeld, Germany).

2.4. In vivo study

The in vivo study was performed using three young pigs (3 months old), weighing approximately 20 kg at the beginning of the study. Young animals were selected to minimize inter-subject variation and due to the similarity of their gut with the same organ in man. Two percent CycA drug nanocrystal suspension and 10% aqueous SLN dispersion (containing 2% CycA) were administered. The microemulsion Sandimmun Neoral/Optoral® was used as reference. Instead of applying a cross-over study design, all three animals were administered with the CycA-loaded SLN dispersion. There was a 12 h fasting prior to administration of each test formulation. The administration of the SLN suspension was followed by a 6-day wash out phase, and then all animals received the Sandimmun® microemulsion. After another 6 days of wash out phase the drug nanocrystal suspension was administered. The CycA dose was 16 mg/kg. All test formulations were diluted with water to yield an administration volume of 40 ml. Prior to the study all pigs were anesthetized and a stomach catheter was positioned by microlaparotomy. Simultaneously a vein catheter was placed in the vena jugular. Blood samples were taken after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12 and 24 h after oral administration. EDTA was added to the blood samples (0.1 μm/ml), which were kept frozen at −20 °C until analysis by the CycA radioimmunoassay. The animals were not fed 12 h before and another 4 h after administration of the formulation. The tested formulations were administered via the stomach catheter. After administration the catheter was flushed with 200 ml water. The pigs were fed with 850 g of a typical standardized food for young pigs 4 h after administration of the test formulation. The in vivo study including the blood analysis was performed at the University Hospital in Pavia/Italy.

2.5. HPLC analysis

HPLC analysis was performed on a Kroma System 2000 (Kontron Instruments, Berlin, Germany) running in the isocratic mode. The system consisted of an HPLC pump 220, an Autosampler T360 and a UV detector 430. Temperature control was performed using a water bath Haake W90 (Haake, Karlsruhe, Germany). Integration of the peaks was performed using the Kontron HPLC software. HPLC analysis was done according to the USP XXII, using an external standard. UV detection was performed at 210 nm using the following parameters: main column Merck LiChroCart 250-4 cartridge, Superspher 100 RP18 (4 μm); guard column MichroCart 4-4, LiChrospher 100 RP18 (5 μm); injection volume of 20 μl; flow rate of 1.3 ml/min; oven temperature of 80 °C; running time of 40 min; and the retention time for CycA was 25–30 min. The mobile phase consisted of 485 parts water, 460 parts acetonitrile, 55 parts *tert*-butyl-methyl-ether and 1 part concentrated phosphoric acid. The method was slightly modified compared to the USP XXII description. The total amount of CycA in the SLN dispersion was determined by dissolving an appropriate amount of SLN dispersion in acetone. The quantity of free CycA (i.e. CycA dissolved in the aqueous dispersion medium) was determined by centrifuging the SLN dispersion over 6 h at 70,000 rpm. The supernatant was used for CycA analysis. The encapsulated amount of CycA was calculated by subtracting the free amount of CycA from the total amount in the dispersion.

3. Results and discussion

In order to incorporate CycA into SLN, the drug needs to possess a sufficiently high solubility in the lipid used for SLN production. Prior to the production of the SLN dispersion a lipid screening was performed by dissolving increasing amounts of CycA in various melted lipids. After dissolution, the lipids were solidified and checked for presence/absence of CycA crystals. Polarized light was used applying a magnification of 630× to search for particles and/or drug crystals larger than 1 μm. At 20% loading of Imwitor®900 no crystals could be microscopically detected supporting that CycA was present as a solid solution (molecularly dispersed in the lipid matrix). This was supported by wide-axis X-ray scattering (WAXS) investigations (Runge, 1998). Based on these observations, aqueous SLN dispersion was produced being composed of 10% lipidic phase (8 parts Imwitor®900 and 2 parts CycA). This means that CycA concentration was 2% with regard to the total SLN dispersion and 20% with regard to the solid lipid phase.

The obtained mean PCS diameter was 157 nm and the PI was 0.42. Typical PI values of emulsions for parenteral nutrition (e.g. Lipofundin®, Intralipid®) are between 0.10 and 0.25 (Müller and Heinemann, 1992). LD analysis yielded a diameter LD50% of 0.30 μm. The diameters LD90% and LD95% were 0.54 and 0.62 μm, respectively. The difference between the PCS diameter and the diameter LD50% can be explained by the different measuring principles, i.e. intensity weighed *z*-average by PCS and volume size distribution based on Fraunhofer diffraction. Despite the different measuring principles, the obtained mean diameters were relatively close. The CycA drug nanosuspension had a PCS diameter of 962 nm and a PI of 0.193. LD50% and LD90% were 1.42 and 1.97 μm, respectively. The CycA content of both SLN dispersion and nanosuspension was verified by HPLC. The encapsulation rate of SLN was found to be 96.1%. This result means that 3.9% of CycA was in the dispersion medium, e.g. solubilized by the surfactant. Drug nanocrystals consist of 100% CycA, therefore no encapsulation rate could be determined.

The solubility of CycA in water is very low being just 0.04 mg/ml at 25 °C; however, it surprisingly increased to 1 mg/ml in physiological sodium chloride solution (0.9%, m/v) at 22 °C (Meinzer et al., 1998). The solubility in the used surfactant mixture (2.5% Tagat®S and 0.5% sodium cholate) was found to be 0.2 mg/ml. However, in the supernatant of CycA-loaded SLN dispersions a maximum solubility of 1.2 mg/ml was found. This is attributed to the fact that a fraction of the surfactant mixture is required to stabilize the SLN particles, which means it is not available anymore for solubilizing CycA. In addition, CycA adheres between the water and the lipid phase, which also affects its concentration in the aqueous dispersion medium.

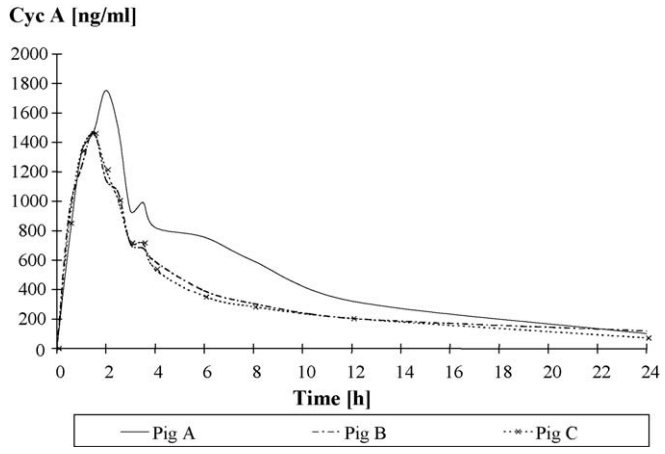

Fig. 1. Blood profiles of the three pigs after administration of the microemulsion Sandimmun Neoral®.

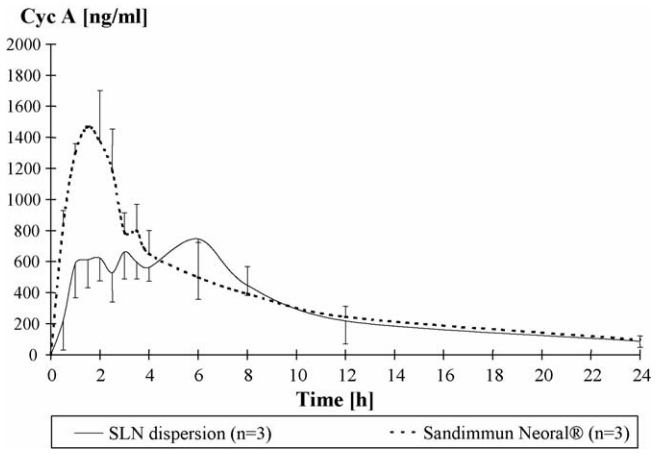

Fig. 2. Mean plasma profiles including standard variations ($n = 3$) after administration of Sandimmun Neoral® vs. CycA-loaded SLN dispersion.

It should also be noted that the solubility of CycA is strongly temperature dependent, which is extremely important for its in vivo administration. The solubility around room temperature is at its maximum value and decreases with increasing temperature, which means it is distinctly lower at body temperature being approximately 17 mg/l in water (Keck et al., 2004a,b). This reduction in solubility can be explained by a conformational change of the aminoacid D-alanin (*R*) configuration being in position 8. It looses hydration water with increasing temperature, thus affecting the CycA conformation and subsequently its solubility.

The blood profiles observed after administration of the microemulsion Sandimmun Neoral® were in agreement with the profiles well known from the literature (Tarr and Yalkowsky, 1989; Lindberg-Freijs and Karlsson, 1994). There was a fast absorption of CycA leading to the plasma peak within approximately the first 2 h after administration. After this initial absorption phase the blood concentration is slowly but steadily decaying. Fig. 1 shows the profiles obtained from the three pigs. Two profiles (pigs B and C) were almost identical.

Administration of CycA-loaded SLN dispersion led to a mean plasma profile with almost similarly low variations, but there was no initial blood peak above 1000 ng/ml as observed for Sandimmun Neoral® (Fig. 2).

Based on the blood concentration data various pharmacokinetic parameters were determined. The parameters were calculated for each pig, giving also the mean values. The side effects of the present microemulsion formulation are caused by too high blood concentrations. Apart from calculating the area under the curve over a period of 24 h ($AUC_{0–24\,h}$) also the areas under the curves were calculated being above the concentration of 800, 1000 and >12,000 ng/ml. The latter three AUC values were calculated in percent of the total $AUC_{0–24\,h}$. In addition, the C_{max} and t_{max} were also obtained. Tables 1 and 2 show the data.

During the performance of the in vivo study, the actual doses of CycA-loaded SLN were calculated as 15.6 mg/kg versus 16.0 mg/kg when administering the reference Sandimmun Neoral®. The absorption of CycA follows a linear kinetic increasing drug concentration/dose (Tarr and Yalkowsky, 1989;

Table 2
C_{max} and t_{max} values of the three pigs including the mean values after administration of CycA-loaded SLN dispersion and Sandimmun Neoral® microemulsion as reference

	C_{max} [ng/ml]		t_{max} [h]	
	SLN	Ref.	SLN	Ref.
Pig A	1193	1753	6	2
Pig B	842	1471	1	1.5
Pig C	814	1460	3	1.5
Average	950	1561	3.3	1.7
S.D.	±211	±166	±2.5	±0.3

Ref. stands for Sandimmun Neoral/Optoral®.

Table 1
Area under the curves (AUC) of the three pigs over a period of 24 h ($AUC_{0–24\,h}$) and areas under the curves at concentrations >1800, >1000 and >1200 ng/ml (calculated for pigs A to C, average and standard deviation (S.D.) are given)

	$AUC_{0–24\,h}$ [ng h/ml]			$AUC_{>800\,ng/ml}$ [%]		$AUC_{>1000\,ng/ml}$ [%]		$AUC_{>1200\,ng/ml}$ [%]	
	SLN	SLN*	Ref.	SLN	Ref.	SLN	Ref.	SLN	Ref.
Pig A	10026	10283	11860	4.8	13.4	1.16	8.2	0	4.8
Pig B	6084	6200	8401	0.3	10.4	0	5.1	0	1.6
Pig C	7224	7409	7926	0	11.5	0	6.1	0	2.3
Average	7778	7964	9396	1.7	11.7	0.4	6.5	0	2.9
S.D.	±2028	±2097	±1540	±2.7	±1.5	±0.7	±1.6	±0	±1.7

Ref. stands for Sandimmun Neoral/Optoral® and SLN* stands for the values obtained for normalized AUC of 16.0 mg/kg dose of cyclosporine in SLN dispersion.

Lindberg-Freijs and Karlsson, 1994). Therefore, when calculating the $AUC_{0-24\,h}$ the difference between the actual administered doses can be extrapolated to obtain a directly comparable AUC (in Table 1, SLN). Table 1 contains the AUC in percent above certain blood concentrations, also given for the single pigs. These data allow the estimation of the risk of undesired side effects, which are known to be nephrotoxicity and hepathotoxicity (Martindale, 1989). The data show that there are differences between the three animals. For example, the pig A shows a distinctly high AUC when treated with different formulations of SLN and microemulsions. This result is in accordance to the fact that there are inter-subject differences when administering CycA orally (Frey et al., 1988). Looking at the mean values in Table 1, the $AUC_{0-24\,h}$ is lower after administering the SLN formulation, which means the two formulations are not bioequivalent. The difference can be explained by the initially high absorption of the microemulsion resulting in the undesired plasma peaks. For example, 11.7% of the total AUC of the microemulsion results from blood levels above 800 ng/ml, only 1.7% of the total AUC of the SLN formulations. Therefore, when comparing both SLN dispersion and microemulsion, the total AUC is not the relevant parameter. Although the duration above the minimum therapeutic concentration is similar for both formulations, it can be stated that the SLN formulation avoids side effects by lacking blood concentrations distinctly higher than 1000 ng/ml. This is evident when looking at the AUC values >1000 and >12,000 ng/ml being 0.4 and 0.0% of the total AUC for SLN, but 6.5 and 2.9% in case of the microemulsion.

The C_{max} of the SLN formulation with 950 ng/ml is distinctly below the 1561 ng/ml observed with the microemulsion. Based on the delayed absorption from the SLN formulation, t_{max} for SLN is 3.3 h compared to 1.7 h for the microemulsion. The latter showed a t_{max} caused by the fast initial plasma peak. Again, C_{max} and t_{max} show inter-subject variations. For both formulations the values of pig A are generally higher compared to pigs B and C. When administering SLN formulation it should be pointed out that no blood concentration above 1200 ng/ml was observed. The absence of such high concentrations results in slightly higher blood levels in the therapeutic window in the time 5–8 h compared to the microemulsion (Fig. 2).

For drug nanocrystal suspensions very pronounced increases in oral bioavailability have been reported for various orally administered drugs (Keck et al., 2004c). In case of CycA drug nanocrystals, the results were rather disappointing, where most of the blood concentrations were in the range between 30 and 70 ng/ml over a period of 14 h. The values were rather low, showing also large differences from measuring time point to time point and also in between the animals, therefore the standard deviations were not plotted (Fig. 2).

The in vitro release of CycA from aqueous SLN dispersion was studied. Due to the low solubility of CycA in water (and resulting analytical problems) an aqueous ethanolic solution of 40% ethanol was used as dissolution medium. The release proved to be relatively fast with 50% released within 15 min (Runge, 1998), revealing that CycA might be preferentially localized in the outer shell of the SLN, similar to Coenzyme Q10 (Dingler, 1998) and acyclovir (Lukowski et al., 1998). Of course, the release was studied without the presence of lipid-degrading enzymes, as they are in the gastrointestinal tract. Based on the obtained in vivo blood profiles, it can be assumed that drug release from the SLN was sufficiently slow to avoid the initial plasma peak but still sufficiently fast enough to reach the therapeutic blood levels. Dissolution of drug nanocrystals is known to be extremely fast. Almost complete dissolution of the CycA nanocrystals in water was observed within 5 min (Keck et al., 2004a,b). According to this observation, it seems possible that the CycA nanocrystals dissolve extremely fast in the gastrointestinal tract, leading to a supersaturated solution. Supersaturated solutions are labile, which means that they tend to change to a thermodynamically more stable system by precipitation of drug microcrystals (with low dissolution velocity) in normally saturated aqueous phase. Therefore, the CycA drug nanocrystals might have dissolved, and precipitated again as slowly dissolving large drug crystals. The consequence was the observed low bioavailability. Another potential explanation is aggregation of the drug nanocrystals in the presence of electrolytes in the gut. It is known in the community that nanocrystal aggregation reduces distinctly the oral bioavailability (however no systematic investigation about this phenomenon has yet been published). Minor aggregation was found to lead to very little changes in the dissolution velocity (Keck et al., 2004a). However, when aggregating CycA nanosuspensions by addiction of NaCl 0.9% (m/v), or especially strongly zeta potential reducing electrolytes such as calcium chloride, the dissolution velocity was strongly reduced.

One – or maybe even both – of these effects might have contributed to the extremely low bioavailability observed with the CycA nanocrystals in vivo.

The absorption of CycA depends very much on the nutritional state of the patient and the presence of bile salts in the gut. In general, it is favourable to have lipid present which induces secretion of bile salts. There are sensors in the duodenum regulating the release of bile salts from the gallbladder, independent on the presence of the fatty acids and triacylglycerols. In addition, the secretion of pancreatic enzymes (lipases) from the pancreatic ducts is regulated. The lipase in combination with co-lipase degrades the triacylglycerols to surface active mono and diacylglycerols. The fatty acids are preferentially cleaved in positions 1 and 3 of the glycerol, there is a steric hindrance of the pancreatic lipase regarding the hydroxyl group in position 2 of the glycerol (Bracco, 1994). The formed surface active mono and diacylglycerols from micelles which can solubilize the drug and promote drug absorption in addition to the bile salts release.

The absorption enhancing effect of lipids on pharmaceutical actives, such as Vitamin E, is very well known (Lukowski et al., 1998; Bracco, 1994). In recent years, the absorption enhancing effect of lipids attracted increasing attention, for example, reflected by the activity of the lipid-based drug delivery group of the American Association of Pharmaceutical Scientists (AAPS). Apart from dissolution and distribution effects of drugs in the gut, the lipid absorption enhancing effect is also an issue when using microemulsions. The company Capsugel® is very much focusing on capsule formulations based on microemulsions to enhance oral bioavailability. Intensive studies of the effect of

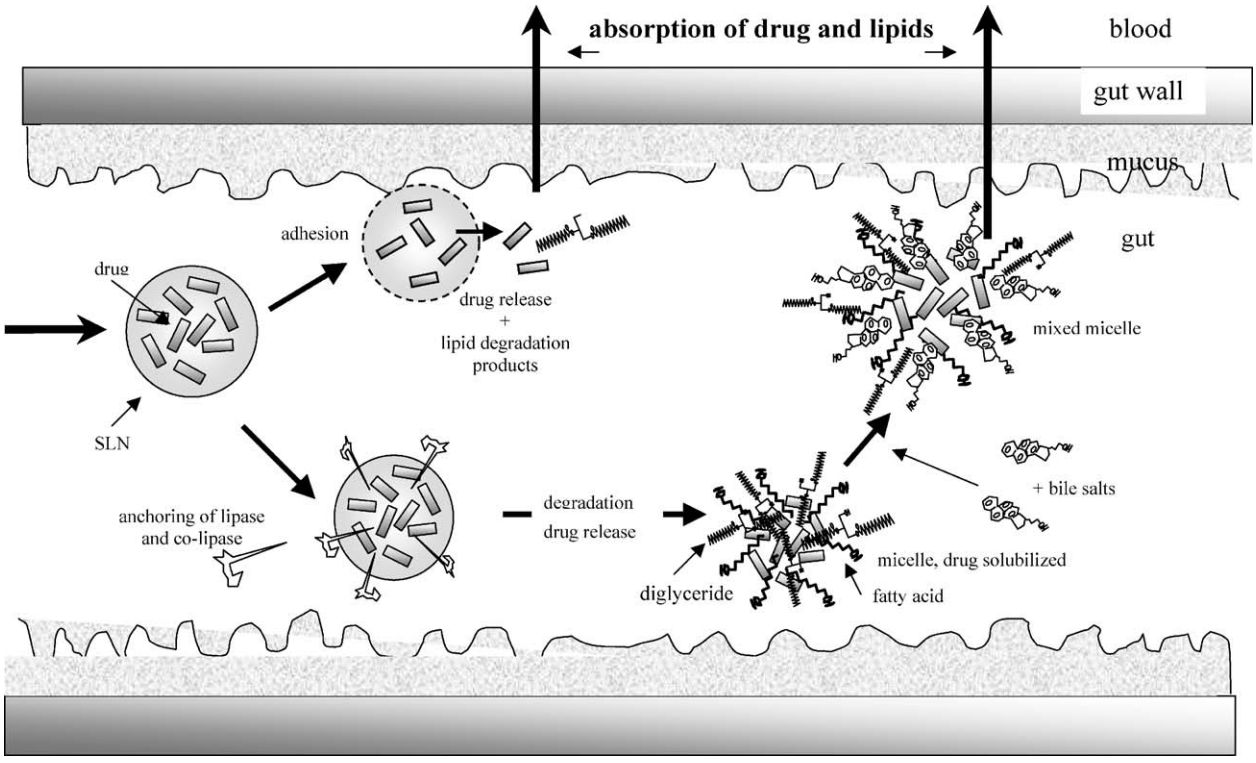

Fig. 3. Absorption enhancing effect of SLN. The particles adhere to the mucosal wall of the gut and the drug molecules are released exactly at the place of absorption. Simultaneously, SLN are degraded by lipases leading to the formation of surface active mono and diacylglycerols solubilizing the drug. Mixed micelles are formed by interaction with bile salts leading finally to the uptake of the lipid and simultaneously drug (so called "mechanism of absorption promotion effect") (with the permission after Müller and Keck, 2004).

lipids on absorption have been performed by the research group of Charman (Charman, 2000; Porter and Charman, 2001; Sek et al., 2002). The basic mechanism can be summarized as shown in Fig. 3. After simultaneous administration of lipid and drug, the lipids are degraded by the enzymes in the gut forming surface active mono and diacylglycerols which can solubilize a poorly soluble drug. Subsequently, interaction with bile salts takes place, leading to the formation of mixed micelles. These mixed micelles promote drug absorption. The lipids taken up simultaneously with the drug are absorbed in a kind of a "mechanism of absorption promotion effect". It should be noted that for a maximum bioavailability enhancing effect, the drug should be closely associated with the lipid. This means that when the lipid is degraded to surface active compounds, the drug should be simultaneously present to be solubilized. The drug should be preferentially dissolved (molecularly dispersed) in the lipid to be digested. Movements of the gastrointestinal tract in combination with the presence of surface active compounds such as bile salts transfer oils and fat in general to a course emulsion (Ritschel et al., 1978). The emulsion droplets are then degraded. Degradation and subsequent solubilization of drugs is faster in case the droplets are finer, preferentially in the nanometer size range and not microdroplets. Both principles, close association of the drug with the lipid and ultrafine dispersion in the nanometer range are realized in the SLN delivery system. Fig. 3 shows the principle mechanisms underlying the absorption enhancing effect of SLN.

Nanoparticles in general possess adhesive properties (Tarr and Yalkowsky, 1989); therefore, the absorption enhancing effect of orally administered nanoparticulate material such as drug nanocrystals and SLN is attributed – apart from additional other factors – to adhesion of the particles to the gut wall. Drug is now released exactly at the place of its absorption, leading to a higher concentration gradient between gut wall and blood. In addition, SLN – in the same manner as other lipids – are degraded by the lipases. Degradation is relatively fast due to the large surface area of the particles. It could be shown that the lipase/co-lipase complex degrades solid lipid particles in a similar mechanism as liquid lipids (Olbrich et al., 2002a,b; Olbrich and Müller, 1999). In case of drug being molecularly dispersed in the solid lipid matrix (solid solution), during the in situ formation of the surface active mono and diacylglycerols the drug being present is solubilized in the acylglycerols micelles. Subsequently, the acylglycerols micelles can directly lead to absorption of lipid and drug as shown in Fig. 3. Alternatively, they can interact with bile salts leading to mixed micelles prior to absorption.

In the present study the optimized blood profile for CycA was obtained just by chance. There were only some orientating in vitro SLN degradation studies. However, it is practically impossible to mimic the complex enzyme composition in the gut in vitro. This means that at the end of the day there is only one way to optimize the degradation of SLN and the release profile, i.e. performing in vivo studies with differently composed lipid

nanoparticles. However, there is a controlled way to prolong or to accelerate SLN degradation and related release. It could be shown that the degradation velocity of the lipid particle matrix depends very much on its chemical composition. Triacylglycerols with shorter fatty acids are degraded faster, in comparison to the ones with long chain fatty acids (Olbrich et al., 2002a). The most important role is played by the stabilizer or stabilizer mixture of the SLN. The stabilizing layer controls the anchoring of the lipase/co-lipase complex. In case of the use of lecithin or sodium cholate as stabilizers, the anchoring of the complex leads to fast degradation. In case sterically stabilizing polymers such as poloxamer are used, there is a stearic hindrance of the anchoring of the complex leading to slower degradation (Olbrich et al., 2002a; Olbrich and Müller, 1999). It could also be shown that intermediate degradation times can be generated in vitro by mixing, for example, lecithin and poloxamer. Therefore, in case the obtained blood profile after oral administration is not optimal, it can be modulated by changing the lipid nanoparticles composition, mainly the stabilizer. It is a very important point that mainly the stabilizing layer controls the particle degradation velocity because there is a certain limitation in exchanging the lipid particle matrix. This matrix needs to provide a sufficiently high solubility of the drug, therefore the number of lipids which can be specifically used for a certain drug is limited.

In case of CycA it should be pointed out that the limited oral bioavailability is not only due to the slow dissolution velocity of the drug/undissolved drug particles in the gut, but also to the presence of an absorption window in the upper gut (Tarr and Yalkowsky, 1989), as well as the potential first pass metabolism that the drug suffers in the gastrointestinal tract (Kolars et al., 1991) and the action of P-glycoprotein transporting CycA back to the gut lumen (Charuk et al., 1995). Intestinal cytochrome P-450 subtype 3A metabolises CycA and thus reducing its activity (Wacher et al., 1998). An additional problem is that increasing concentrations of CycA lead to increased activity of the cytochrome system. Looking at the location of P-glycoprotein and the intestinal cytochrome, it seems to be that the P-glycoprotein is preferentially located in the upper gastrointestinal tract, while the intestinal cytochrome is more in the lower gastrointestinal tract. This means that CycA is transported back to the gut lumen in the upper tract and it will subsequently be metabolized during the absorption process in the lower gut by the cytochrome. Thus, to further enhance the oral bioavailability of the SLN delivery system, it would be beneficial to simultaneously incorporate, for example, a P-glycoprotein inhibitor. Sodium cholate was chosen as stabilizer for SLN because it is known to play a role in the CycA absorption. For further improvement of the formulation it might be sensible to use a stabilizer mixture with Tween 80 because this latter is also known as P-glycoprotein inhibitor (Zhang et al., 2003).

4. Conclusions

SLN proved to be a suitable oral delivery system to enhance the oral bioavailability of the model drug CycA. Although, in the present study, these carriers did not reduce the variability in oral absorption, the release of CycA by diffusion from the particle matrix and release by degradation of the matrix by enzymes was in a range that an optimized blood profile was obtained, i.e. the concentrations were in the therapeutic range over a period similar to the microemulsion, but simultaneously avoiding the undesired plasma peak. In this study, CycA nanocrystals failed to achieve a sufficiently high bioavailability. Potential reasons were discussed above. It is believed that the lipids present in the SLN system play an important role in the absorption enhancing effect. Drug nanocrystals represent an optimal solution for many poorly soluble substances and, therefore, they are still considered as a formulation of first choice due to the simplicity of the system. However, in case they fail the SLN are the alternative delivery systems utilizing the absorption enhancing effects of lipids. The dissolution velocity of drug nanocrystals, and the resulting blood profiles can be modulated only to a limited extent. The dissolution velocity is compound-specific (depends on the dissolution pressure) and can be varied to some extent by the particle size (surface area). In contrast, the presence of a matrix in the SLN system gives more flexibility in modulating drug release by diffusion and lipid matrix degradation. From this, the SLN provide definitely more flexibility in modulation of release and blood profiles. To summarize, both the drug nanocrystals and SLN can be considered as complementary oral drug delivery systems.

References

Beveridge, T., Gratwohl, A., Michot, F., Niederberger, W., Nüesch, E., Nussbaumer, K., Schaub, P., Speck, B., 1981. Cyclosporine: pharmacokinetics after a single dose in man and serum levels after multiple dosing in recipients of allogenic bone-marrow grafts. Curr. Ther. Res. 30, 5–18.

Bracco, U., 1994. Effect of triglycerides structure on fat absorption. Am. J. Clin. Nutr. 60, 1002S–1009S.

Charuk, J.H., Wong, P.Y., Reithmeier, R.A., 1995. Differential interaction of human renal P-glycoprotein with various metabolites and analogues of cyclosporin A. Am. J. Physiol. 269, F31–F39.

Charman, W.N., 2000. Lipids, lipophilic drugs, and oral drug delivery—some emerging concepts. J. Pharm. Sci. 89, 967–978.

Dingler, A., 1998. Feste Lipid-Nanopartickel als kolloidale Wirkstoffträgersysteme zur dermalen Applikation. Ph.D. Thesis. Freie Universität Berlin, Berlin.

Fernandes, C.M., Ramos, P., Falcão, A.C., Veiga, F.J., 2003. Hydrophilic and hydrophobic cyclodextrins in a new sustained release oral formulation of nicardipine: in vitro evaluation and bioavailability studies in rabbits. J. Control. Release 88, 127–134.

Fichera, M., Keck, C., Müller, R.H., 2004. Nanopure® Technology – Drug Nanocrystals for the Delivery of Poorly Soluble Drugs, Particles 2004 – Particle Synthesis, Characterization and Particle-Based Advanced Materials. Orlando, FL.

Frey, F.J., Horber, F.F., Frey, B.M., 1988. Through levels and concentration of cyclosporine in patients undergoing renal transplantation. Clin. Pharm. Ther. 43, 55–62.

Irngartinger, M., Camuglia, V., Damm, M., Goede, J., Frijlink, H.W., 2004. Pulmonary delivery of therapeutic peptides via dry powder inhalation: effects of micronisation and manufacturing. Eur. J. Pharm. Biopharm. 58, 7–14.

Keck, C., Fichtinger, A., Viernsten, H., Müller, R.H., 2004a. Production and optimisation of oral cyclosporine nanocrystals. In: AAPS Annual Meeting, Baltimore.

Keck, C., Fichtinger, A., Viernsten, H., Müller, R.H., 2004b. Oral drug nanocrystals—effect of potential aggregation on bioavailability. In: AAPS Annual Meeting, Baltimore.

Keck, C.M., Bushrab, N.F., Müller, R.H., 2004c. Nanopure® nanocrystals for oral delivery of poorly soluble drugs. In: Particles. Orlando.

Kolars, J.C., Awni, W.M., Merion, R.M., Watkins, P.B., 1991. First-pass metabolism of cyclosporine by the gut. Lancet 338, 1488–1490.

Lindberg-Freijs, A., Karlsson, M.O., 1994. Dose dependent absorption and linear disposition of cyclosporine A in rat. Biopharm. Drug Dispos. 15, 75–86.

Liversidge, G., 2003. Milling Methods—NanoCrystals™, IIR Drus Delivery Partnerships™ Meeting, Workshop Nanotechnology—Solid Particles, Lipids and Nanocomplexes. Cologne/Germany.

Lukowski, G., Werner, U., Pflegel, P., 1998. Surface investigation and drug release of drug-loaded solid lipid nanoparticles. In: Proceedings of the 2nd World Meeting APGI/APV, Paris, pp. 573–574.

Martindale, 1989. The Extra Pharmacopoeia, 34th ed. The Pharmaceutical Press, London, pp. 614–619.

Meinzer, A., Müller, E., Vonderscher, J., 1998. Perorale Mikroemulsionsformulierung—Sandimmun Optoral®/Neoral®. In: Müller, R.H., Hildebrandt, G.E. (Eds.), Pharmazeutische Technologue: Moderne Arzneiformen. Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp. 169–177.

Moschwitzer, J., Müller, R.H., 2006. Spray coated pellets as carrier system for mucoadhesive drug nanocrystals. Eur. J. Pharm. Biopharm. 62, 282–287.

Moschwitzer, J., Achleitner, G., Pomper, H., Muller, R.H., 2004. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur. J. Pharm. Biopharm. 58, 615–619.

Müller, R.H., 2001. Dispersions for the Formulation of Slightly or Poorly Soluble Agents. DE 100 36 871.9 (2000), PDT Application PCT/EP01/08726, Germany.

Müller, R.H., Heinemann, S., 1992. Fat emulsions for parenteral nutrition. I. Evaluation of microscopic and laser light scattering methods for the determination of the physical stability. Clin. Nutr. 11, 223–236.

Müller, R.H., Keck, C.M., 2004. Challenges and solutions for the delivery of biotech drugs—a review of drug nanocrystal technology and lipid nanoparticles. J. Biotechnol. 113, 151–170.

Müller, R.H., Souto, E.B. Lipid nanoparticles (SLN and NLC) for drug delivery. In: Kumar, R., Tabata, Y., Domb, A. (Eds.), Nanoparticles for Pharmaceutical Applications, American Scientific Publishers, 2006.

Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19.

Müller, R.H., Mäder, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of art. Eur. J. Pharm. Biopharm. 50, 161–177.

Müller, R.H., Mäder, K., Lippacher, A., Jenning, V., 1998a. Fest-flüssig (halbfeste) Lipidpartikel und Verfahren zur Herstellung hochkonzentrierter Lipidpartikeldispersionen. PCT Application PCT/EP00/04565.

Müller, R.H., Runge, S., Ravelli, V., 1998b. Pharmaceutical Cyclosporin Formulation of Improved Biopharmaceutical Performance and Improved Physical Quality and Stability and Process for Producing Same. Deutsche Patentanmeldung 198 19 273 A1.

Müller, R.H., Mehnert, W., Lucks, J.-S., Schwarz, C., zur Mühlen, A., Weyhers, H., Freitas, C., Rühl, D., 1995. Solid lipid nanoparticles (SLN)—an alternative colloidal carrier system for controlled drug delivery. Eur. J. Pharm. Biopharm. 41, 62–69.

Olbrich, C., Müller, R.H., 1999. Enzymatic degradation of SLN—effect of surfactants and surfactant mixtures. Int. J. Pharm. 180, 31–39.

Olbrich, C., Kayser, O., Müller, R.H., 2002a. Enzymatic degradation of Dynasan 114 SLN—effect of surfactants and particle size. J. Nanoparticle Res. 4, 121–129.

Olbrich, C., Kayser, O., Müller, R.H., 2002b. Lipase degradation of Dynasan 114 and 116 solid lipid nanoparticles (SLN)—effect of surfactants, storage time and crystallinity. Int. J. Pharm. 237, 119–128.

Penkler, L., Müller, R.H., Runge, S., Ravelli, V., 1999. Pharmaceutical Cyclosporin Formulation with Improved Biopharmaceutical Properties, Improved Physical Quality and Greater Stability, and Method for Producing Said Formulation. PCT Application PCT/EP99/02892.

Porter, C.J., Charman, W.N., 2001. In vitro assessment of oral lipid based formulations. Adv. Drug Deliv. Rev. 50, S127–S147.

Rasenack, N., Hartenhauer, H., Muller, B.W., 2003. Microcrystals for dissolution rate enhancement of poorly water-soluble drugs. Int. J. Pharm. 254 (2), 137–145.

Ritschel, W.A., Ritter, S.M., Beiser, C.R., 1978. Are suppository bases absorbed? Study on peroral and rectal absorption of lipids in rabbits. Pharm. Ind. 40, 82–88.

Runge, S., 1998. Feste Lipidnanopartikel (SLN®) als kolloidaler Arzneistoffträger für die orale Applikation von Ciclosporin A. Ph.D. Thesis. Freie Universität Berlin, Berlin.

Sek, L., Porter, C.J., Kaukonen, A.M., Charman, W.N., 2002. Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. J. Pharm. Pharmacol. 54, 29–41.

Souto, E.B., 2005. SLN and NLC for topical delivery of antifungals. Ph.D. Thesis. Freie Universität Berlin, Berlin.

Sridevi, S., Chauhan, A.S., Chalasani, K.B., Jain, A.K., Diwan, P.V., 2003. Enhancement of dissolution and oral bioavailability of gliquidone with hydroxy propyl-beta-cyclodextrin. Pharmazie 58, 807–810.

Tarr, B.D., Yalkowsky, S.H., 1989. Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size. Pharm. Res. 6, 40–43.

Vonderscher, J., Meinzer, A., 1994. Rationale for the development of Sandimmune Neoral. Transplant. Proc. 26, 2925–2927.

Wacher, V.J., Silverman, J.A., Zhang, Y., Benet, L.Z., 1998. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. J. Pharm. Sci. 18, 1322–1330.

Zhang, H., Yao, M., Morrison, R.A., Chong, S., 2003. Commonly used surfactant, Tween 80, improves absorption of P-glycoprotein substrate, digoxin, in rats. Arch. Pharm. Res. 26, 768–772.

zur Mühlen, A., Schwarz, C., Mehnert, W., 1998. Solid lipid nanoparticles (SLN) for controlled drug delivery—drug release and release mechanism. Eur. J. Pharm. Biopharm. 45, 149–155.