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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*

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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).

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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