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Research paper

Supersaturated polymeric micelles for oral cyclosporine A delivery

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ABSTRACT

Polymeric micelles provide a promising platform for improving oral absorption of poorly soluble drugs. However, improved understanding of how drug retention within the hydrophobic micelle core can reduce drug absorption is required. We designed supersaturated polymeric micelles (Super-PMs) to increase molecularly dissolved drug concentration and gain an insight into the effect of the degree of supersaturation on oral absorption of cyclosporine A (CsA) in rats. The drug release from Super-PMs increased with an increase in initial supersaturation degrees in micelles. The cellular uptake of coumarin-6 was reduced by the retention of drug in polymer micelles. The transport flux of CsA across Caco-2 monolayer was increased with initial supersaturation degrees of 0.81–3.53 ($p < 0.05$). However, increase in supersaturation to 5.64 actually resulted in decreased CsA transport. The same trend was observed in a rat *in vivo* absorption study, in which the highest bioavailability of 134.6 ± 24.7% (relative to a commercial product, Sandimmun Neoral®, $p < 0.01$) was achieved when the supersaturation degree was 3.53. These results demonstrated that Super-PMs were a promising drug delivery system for compounds with low aqueous solubility. This study also provided an experimental proof for the hypothesis that moderately supersaturated formulations are valuable alternative to high supersaturation formulations, resulting in optimal *in vivo* performance, and the degree of supersaturation should be carefully controlled to optimize drug absorption.

1. Introduction

Many drugs developed in recent years are poorly water-soluble. The poor solubility is the main barrier to oral absorption of these drugs. Many strategies have been employed to improve drug solubility, including cyclodextrin complex formation [1], amorphous formulations [2,3], and lipid-based formulations [4]. Over the past decade, polymeric micelles (PMs) have been investigated as promising delivery system for the poorly soluble drugs [5–7]. Classically defined PMs, formed using amphiphilic polymers, possess a hydrophobic core, which acts as a reservoir for lipophilic molecules, surrounded by a hydrophilic corona exposed to the aqueous environment. The corona confers aqueous solubility and steric stability to micelle assemblies [8].

Recent studies involving PMs have focused on the effect of micelle solubilization on drug permeability and *in vivo* absorption; however, less attention has been paid to the thermodynamic activity of drugs in polymer micelles. It is commonly thought that poorly soluble drugs can be readily absorbed once they are incorporated into micelles. However, some *in vitro* and *in vivo* studies have yielded contradictory data. For example, Poloxamer 407 micelle solubilized carbamazepine solution exhibited poor permeation across Caco-2 monolayer than the supersaturated solution achieved by hydroxypropyl methylcellulose acetate succinate (HPMC-AS) solid dispersion [9]. The loading of risperidone into PEG-b-P(CL-co-TMC) micelles did not improve its permeability across Caco-2 monolayer [10]. Moreover, in some cases, PMs have been found to impede the absorption of hydrophobic drugs [11,12]. For example, Cremophor EL micelles have been shown to impair absorption of paclitaxel [12]. The gastrointestinal absorption of highly lipophilic compounds from PMs is largely related to the

Abbreviations: CsA, cyclosporine A; CsD, cyclosporine D; P-gp, P-glycoprotein; PMs, polymeric micelles; HPMC-AS, hydroxypropyl methylcellulose acetate succinate; DAPI, 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride; LC, loading content; EE, entrapment efficiency; TEM, transmission electron microscopy; PCS, photon correlation spectroscopy; HBSS, Hank's balanced salt solution; Rh123, rhodamine 123; LS, low Soluplus® concentration; HS, high Soluplus® concentration; CR, coumarin-6-loaded Cremophor RH40 micelles; CS, coumarin-6 reference solution; Verap, verapamil; AP, apical; BL, basolateral; LOD, limit of detection; LOQ, limit of quantification; C_{max}, maximal blood concentration of the drug; T_{max}, time taken to reach the maximum blood concentration; AUC_{0-24h}, area under the blood drug concentration–time curve; RBA, relative oral bioavailability; Tf, transport flux.

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concentration of the free drug, and the uptake of intact micelles through pinocytosis is in fact a minor route of drug absorption [13]. The conflicting results could be attributed to the unpredictable thermodynamic activity of the drug molecules in solution because of micellar solubilization and retention within the carriers. As a result, the conditions that cause micelle dissociation and drug release upon dilution *in vivo* cannot be predicted accurately *in vitro*. Therefore, when developing oral PM formulations for poorly soluble drugs, it is important to increase the thermodynamic activity of drug in order to achieve desirable oral drug absorption.

Supersaturated drug delivery systems [14], including solid dispersions [15], amorphous drug nanoparticles [16,17], and supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS) [18], have been thoroughly investigated as routes to increase drug thermodynamic activity. However, to our knowledge, this has never been investigated using PMs. Commonly used micelle solubilizing formulations attempt to avoid the creation of a supersaturated state by increasing the solubilizing capacity of the gastrointestinal environment [14]. This approach merely solubilizes drugs by incorporating them into colloidal species. The free drug fraction, in equilibrium with the solubilized fraction, is still limited by its poor aqueous solubility. The use of Super-PMs provides a micelle solution containing a drug concentration in excess of that required for equilibrium to be reached, resulting in a supersaturated state [19]. Super-PMs can be prepared using the hot melt extrusion-hydration method. When drugs are administered as a Super-PM solution, drug thermodynamic activity is greatly increased, compared with commonly used micelles prepared by direct dissolution of the drug in an empty micelle solution or direct dialysis method [20]. Micelles prepared by direct dissolution or dialysis methods have a low amount of drug incorporated and show limited release of free drug from the micelle particles [7,21,22]. However, Super-PMs are thermodynamically unstable, as the supersaturated drug has a higher energy than the crystalline drug does. Once supersaturation has been induced, drug molecules have a tendency to recrystallize, and the higher the degree of supersaturation, the lower the physical stability, leading to an increased tendency for drug precipitation. Therefore, in order to benefit from the supersaturated state, drug activity should be kinetically or thermodynamically controlled to avoid either precipitation upon dilution or sequestration within the micellar phase, because both of these could lead to incomplete drug absorption [14,23].

The aim of the present study was to investigate the ability of Super-PMs to enhance oral bioavailability of a poorly soluble drug and most importantly, to gain an insight into the effect of the degree of supersaturation on its *in vivo* oral absorption in rats. Cyclosporine A (CsA) was selected as the model drug. CsA is a highly effective immunosuppressive agent, which is widely used clinically for prevention of allograft rejection after organ transplantation and treatment of autoimmune disease [24]. The oral bioavailability of CsA is low and irregular due to its low water solubility (7 μg/mL at 37 °C) [25], its large molecular weight (1202 Da), and being a substrate of P-glycoprotein (P-gp). Soluplus®, a graft amphiphilic polymer (Fig. 1) newly introduced by BASF, was selected for the development of Super-PMs using the hot melt extrusion-hydration method. Soluplus® has a lower critical micelle concentration (CMC) of 7.6 mg/L in water (23 °C, data on file, BASF, Pharma Ingredients & Services, Germany; Technical Information 2009) than other polymers, e.g. Tocopherol Acid Polyethylene Glycol1000 Succinate (TPGS), poloxamer 407, PEG-b-PLA, Cremophor EL [26,27], thus, can easily form colloidal micelles with good solubilization capacity for poorly soluble drugs [28]. In addition, the superordinary viscosity above the glass transition temperature of about 70 °C makes it be so fit for developing solid solutions by hot melt extrusion before the preparation of PMs solution [29,30].

2. Materials and methods

2.1. Materials

CsA and cyclosporine D (CsD) were purchased from Fujian Kerui Pharmaceutical Co., Ltd. (Fujian, China). Soluplus® and Cremophor RH40 were kindly donated by BASF (Ludwigshafen, Germany). Sandimmun Neoral® soft gelatin capsules (25 mg strength) were purchased from Novartis (Bern, Switzerland). Coumarin-6 and rhodamine-123 (Rh123) were purchased from Sigma–Aldrich (Saint Louis, Missouri, USA). Verapamil (Verap) was purchased from Tianjin Central Pharmaceutical Factory (Tianjin, China). RPMI 1640 medium and 0.25% trypsin/0.53 mmol/L EDTA were purchased from Invitrogen (Ontario, CA). Fetal bovine serum was obtained from Sijiqing Biological Engineering Materials Co. Ltd. (Zhejiang, China). Penicillin, streptomycin, and 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Paraformaldehyde, heparin sodium (>150 IU/mg), and isoflurane were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetonitrile, methanol, *n*-hexane, and *tert*-butyl methyl ether of HPLC grade were purchased from Tedia Company Inc. (Fairfield, Ohio, USA). Water was obtained from a Milli-Q water purification system (Millipore Corp., Billerica, Massachusetts, USA). All other chemicals were of analytical reagent grade.

2.2. Methods

2.2.1. Phase solubility studies

To evaluate the solubilizing ability of Soluplus®, the phase solubility of CsA in distilled water containing various Soluplus® concentrations (ranging from 0.00% to 6.00%, w/v) was measured at various temperatures (15, 25, and 37 °C). The measurements were performed in triplicate using the method reported by Higuchi [31] and Mehanna [32]. Briefly, excess CsA was added to each Soluplus® solution in a glass vial to form a suspension. The vial was sealed and kept in a biological shaker (SPH-100F, Shanghai, China) at 100 rpm for 48 h. The suspension was then passed through a 0.45-μm mixed cellulose ester membrane (Sinopharm Chemical Reagent Co., China). Five milliliters of initial filtrate was discarded, and the subsequent filtrate was diluted with methanol before determining the concentration of CsA in the filtrate using a Shimadzu series HPLC system (Model SIL-20A, Shimadzu, Japan) equipped with a pump (LC-20AT), a diode array detector (SPD-M20A), and a work station (Shimadzu liquid chromatography). The analytical column was ZORBAX Eclipse XDB-C18 (5 μm,

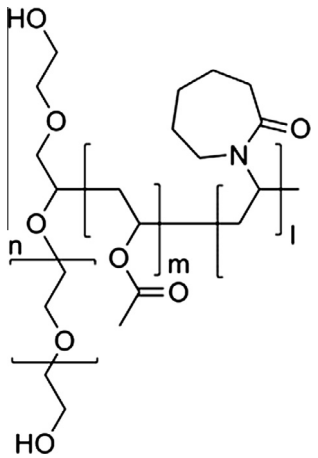

Fig. 1. Molecular structure of Soluplus®.

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