European Journal of Pharmaceutical Sciences 49 (2013) 864–872

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Highly ordered mesoporous carbon nanomatrix as a new approach to improve the oral absorption of the water-insoluble drug, simvastatin

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ARTICLE INFO

Article history:
Received 23 April 2013
Received in revised form 27 May 2013
Accepted 30 May 2013
Available online 18 June 2013

Keywords:
Nanomatrix
Mesoporous carbon
Water-insoluble drugs
Solubility
Crystalline state
Bioavailability

ABSTRACT

Three different kinds of highly ordered mesoporous carbon (HMC) matrices with different morphologies (hexagonal, spherical and fibrous), particle sizes (700 nm, 400–900 nm and 1–4 μm) and pore diameters were compared as drug carriers for a model drug, simvastatin (SIM). The physicochemical properties of the SIM-loaded composites were studied using field emission scanning electron microscopy (FESEM), specific surface area analysis, differential scanning calorimetry (DSC), wide-angle X-ray scattering (WAXS), HPLC, solubility measurement and dissolution testing. Furthermore, the oral bioavailability of SIM-loaded SHMC (spherical HMC nanomatrix) in beagle dogs was compared with that of the reference formulation (Zocor®). The results obtained showed that SIM molecules are encapsulated in a noncrystalline state due to geometric confinement in the nanopores of HMC. *In vitro* dissolution testing showed that the dissolution rate of SIM released from monodispersed SHMC was significantly faster compared with that of crystalline SIM and other SIM-loaded composites. In addition, *in vivo* bioavailability study demonstrated that the relative bioavailability of SIM and SIM β-hydroxy acid (an active metabolite of SIM) for SIM-loaded SHMC formulation was 138.42% and 163.55%, respectively. In conclusion, monodispersed SHMC appear to be a more promising candidate as a new oral drug delivery vehicle providing a rapid drug release and enhanced oral bioavailability.

1. Introduction

The poor aqueous solubility and insufficient dissolution of many active pharmaceutical ingredients are a major technical problem, especially for pharmaceutical researchers involved in formulation development (Blagden et al., 2007). It is estimated that 40% of new chemical entities are poorly soluble or insoluble in water, and up to 50% of orally administered drugs have formulation problems related to low bioavailability (Lipinski et al., 1997). In order for a drug to be absorbed into the systemic circulation following oral administration, it must be dissolved in gastrointestinal fluids (Hörter and Dressman, 2001). For many hydrophobic active pharmaceutical ingredients that cross the gastrointestinal mucosa easily, the drug levels achieved will be dictated by the time required for the dosage form to release its contents, and for the drug to dissolve (Huanga and Tong, 2004; Manly et al., 2007). Hence, improving the saturation solubility and dissolution rate of water-insoluble drugs is very important and presents a major challenge to formulation scientists seeking to obtain complete absorption of active pharmaceutical ingredients (Vasconcelos et al., 2007).

Over the years, the major formulation tools employed to increase the dissolution rate and saturation solubility include: the use of solid dispersions (Vasconcelos et al., 2007), nanosizing (Kesisoglou et al., 2007), inclusion complexes (Sadighi et al., 2012), and salt forms of active pharmaceutical ingredients (Bastin et al., 2000). Another, alternative technique is to prepare pharmaceutical agents in an amorphous form. The amorphous form is a high-energy state that exhibits increased saturation solubility and dissolution rate and, thus, enhanced oral bioavailability (Manly et al., 2007; Vasconcelos et al., 2007). One strategy that is increasingly popular as a means of stabilizing amorphous drugs is adsorption in the nanopores of mesoporous materials (Ambrogi et al., 2008; Barbé et al., 2004; Charnay et al., 2004; Heikkilä et al., 2007; Thomas et al., 2009). Although a variety of chemically different mesoporous matrices have been reported, mesoporous silica is the most widely investigated in the field of drug delivery (Kim et al., 2011; Tan et al., 2011; Jaganathan and Godin, 2012; Tang et al., 2012; Wani et al., 2012; Zhang et al., 2012). In recent years, there has been significant interest in the development of mesoporous carbon materials with a uniform pore structure. Highly ordered mesoporous carbon (HMC) can be synthesized by the nanocasting

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http://dx.doi.org/10.1016/j.ejps.2013.05.031

technique using highly ordered mesoporous silica (HMS) as a template (Liu et al., 2006, 2008). HMC has a larger pore volume and higher specific surface area compared with other mesoporous materials (zeolite, silica, aluminum oxide, etc.) (Liang et al., 2008; Vashist et al., 2011) and, so, it should have a higher drug loading capacity. In addition to the nanocavities of HMC being able to change the crystalline state of a drug to an amorphous one (Wani et al., 2012), the nanocavities also effectively restrict drug recrystallization and significantly reduce the particle size of the amorphous drug (Qian and Bogner, 2011). All these properties demonstrate the potential and advantages of using HMC in oral drug delivery systems. However, few *in vitro* studies have been conducted using oral drug delivery systems involving HMC carriers (Liang et al., 2008; Li et al., 2011; Vashist et al., 2011; Wang et al., 2011) and, to the best of our knowledge, there are no reports of the use of HMC nanomatrix for oral drug delivery *in vivo*.

Based on the above considerations, we have designed three kinds of HMC matrices as oral drug carriers and loaded a model water-insoluble drug into the nanopore channels. SIM is a cholesterol-lowering agent which is widely used to treat hypercholesterolemia (Illingworth and Tobert, 2000). SIM has a poor solubility (6.3 μg/ml, pH 1–7, at 25 °C) and is, thus, a good model drug for formulation in the amorphous state to enhance its dissolution rate (Graeser et al., 2008). The purpose of the present study was to develop and investigate the properties and mechanism of enhancement of dissolution and oral bioavailability of the SIM-loaded HMC. To achieve this aim, the effects of different morphologies, particle sizes and pore diameters of HMC on the drug loading and release behaviors of the model drug SIM were systematically studied using FESEM, transmission electron microscopy (TEM), specific surface area analysis, DSC, WAXS, HPLC, solubility measurement and dissolution testing. Furthermore, pharmacokinetic profiling of SIM after oral administration of SIM-loaded SHMC or the commercial product Zocor® in beagle dogs was made with use of ultraperformance liquid chromatography equipped with electrospray ionization mass spectrometry (UPLC/ESI-MS). The results obtained showed that monodispersed SHMC nanomatrix is a promising carrier able to improve the dissolution rate and subsequently the oral absorption of the water-insoluble drug SIM.

2. Materials and methods

2.1. Chemicals

SIM (USP grade, ⩾98%), Lovastatin (LOV, ⩾99%) and SIMA ammonium salt were kindly donated from Hisun Pharma (Zhejiang, China). Guaranteed-grade sucrose, tetraethyl orthosilicate, HPLC-grade acetonitrile and ethyl acetate were supplied from Kemiou (Tianjin, China). Pluronic 123 was obtained from BASF (Ludwigshafen, Germany). SIM tablets (Zocor®) were obtained from Merck Sharp & Dohme (Hangzhou, China). Lactose monohydrate and pregelatinized starch were donated from Roquette (Jiangsu, China). Avicel® PH microcrystalline cellulose (MCC) and croscarmellose sodium were supplied from FMC BioPolymer (Philadelphia, PA, USA). All other reagents were of reagent grade and were used as purchased without further purification.

2.2. Preparation of HMS nanomatrix and micromatrix

A series of HMS matrices were synthesized according to procedures described in the literature with some modifications, using tetraethyl orthosilicate as a silicate source. For the synthesis of hexagonal HMS (HHMS) nanomatrix (Wang et al., 2009a,b), 2.0 g glycerol and 2.0 g Pluronic 123 were dissolved in 76 ml 2.5 M HCl solution at ambient temperature. Then 4.2 g tetraethyl orthosilicate was added to the Pluronic 123 solution. The stirring was allowed to continue for 10 min before switching to static synthesis conditions at 38 °C. After 24 h, the resulting gel mixture was taken to a Teflon-coated stainless-steel autoclave and kept for approximately 20 h at 80 °C. The resulting precipitate was recovered, washed, and dried. Finally, the solid product was calcined in air at 600 °C for 5 h. Spherical HMS (SHMS) nanomatrix (Liu et al., 2009) were synthesized in the same way as HHMS, except for the use of 0.33 g cetrimonium bromide as a co-template and 76 ml 1.5 M HCl solution. To prepare fibrous HMS (FHMS) micromatrix (Zhao et al., 1998), 2.0 g Pluronic 123 was dissolved in 76 ml 1.75 M HCl solution. Subsequently, 4.2 g tetraethyl orthosilicate was added to this solution with stirring at 38 °C for 24 h and the synthesized material was filtered, washed, and calcined at 600 °C for 5 h.

2.3. Preparation of HMC nanomatrix and micromatrix

Three different kinds of HMC matrices with different morphologies and particle sizes were replicated from the HMS templates using sucrose as the carbon precursor (Lee et al., 2008). In a typical experiment, a precursor solution, containing 0.26 g boric acid, 0.14 g H_2SO_4, 1.25 g sucrose and 4.0 ml purified water was allowed to infiltrate the nanocavities of the respective HMS template (1.0 g HHMS, SHMS and FHMS, respectively). Following this, the mixture was dried at 100 °C for 6 h in a DGT-10 drying oven (Haier, China), then the temperature was increased to 160 °C and held there for approximately 6 h. The infiltration and drying procedure were repeated again with an additional 3.68 g precursor solution, before the silica/sucrose composite was carbonized at 900 °C for 3 h under a nitrogen purge of 60 ml/min. The silica template was removed by treatment with 10% HF solution for 24 h. Finally, the respective HMC product (HHMC, SHMC and FHMC, respectively) was filtered, washed with ethanol, and dried in a vacuum oven. SHMC-2 was synthesized in the same way as SHMC but without adding boric acid. *In* vitro cytotoxicity of HMC on Caco-2 cells was assessed by the MTT reduction assay. The results of MTT assay indicated that the prepared HMC exhibited a very low cytotoxicity (specific data can be found in Fig. S1 in Supplementary Data).

2.4. Loading SIM into the porous materials

A solvent immersion/evaporation procedure was used to load the porous materials with SIM. In a typical experiment, 0.6 g HMC (HHMC, SHMC, SHMC-2 and FHMC, respectively) was added to 20 ml 0.34 M SIM solution. Ethanol was used as the loading solvent. Then, the particle suspensions were ultrasonicated for a few minutes and kept gentle stirring on a HJ-A6 magnetic stir plate (Runhua, China) for 24 h to achieve maximum adsorption in the nanocavities of HMC. The adsorption procedure was protected from the light. Subsequently, the SIM-loaded particles were separated from the drug solution by filtration under vacuum. The moist powder obtained was dried at 40 °C under reduced pressure (10^{-3} bar) for 6 h to remove any residual ethanol from the nanocavities of HMC. The drug-loaded samples were referred to as SIM-HHMC, SIM-SHMC, SIM-SHMC-2 and SIM-FHMC, respectively. The same process was repeated for HHMS, SHMS and FHMS. In addition, the SIM-SHMC tablets were prepared by a process of direct compression with physical blending of SIM-SHMC (20 mg, expressed as SIM equivalents), lactose monohydrate, MCC (PH-102), pregelatinized starch, MCC (PH-200), croscarmellose sodium and magnesium stearate (in a mass ratio of 20/142/24/26/39.5/9.2/4.5).