Colloids and Surfaces B: Biointerfaces 128 (2015) 77–85

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Development of novel mesoporous nanomatrix-supported lipid bilayers for oral sustained delivery of the water-insoluble drug, lovastatin

Yanzhuo Zhang[a,*], Heran Zhang[b], Erxi Che[c], Lihua Zhang[a], Jin Han[a], Yihua Yang[a], Siling Wang[c], Miao Zhang[d], Cunqiang Gao[d]

[a] Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical College, Xuzhou 221004, China
[b] Centre for Pharmaceutical Preparations Technology & Research, Tianjin Institute of Pharmaceutical Research, Tianjin 300193, China
[c] Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China
[d] Pharmaceutical Division, Jiangsu Hengrui Medicine Co. Ltd., Lianyungang 222047, China

ARTICLE INFO

Article history:
Received 28 September 2014
Received in revised form
13 December 2014
Accepted 11 February 2015
Available online 19 February 2015

Keywords:
Drug delivery
Amorphous state
Sustained release
Oral absorption
Cytotoxicity

ABSTRACT

The purpose of this study was to investigate the effect of a core/shell structured nanocomposite, mesoporous nanomatrix-supported lipid bilayer (MN-SLB), as an oral drug nanocarrier, on the dissolution behavior and *in vivo* absorption of a water-insoluble drug, lovastatin (LOV). The formulation strategy was based on the use of drug-loaded mesoporous silica as the core for the fusion of liposomes. Field emission scanning electron microscopy (FESEM), cryogenic transmission electron microscopy (Cryo-TEM) and nitrogen adsorption were used to systematically characterize the drug carrier and drug-loaded MN-SLB formulation, confirming the successful inclusion of LOV into the nano-pores of MN-SLB. Powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) confirmed that the incorporated drug in the carrier was in an amorphous state. An *in vitro* dissolution study showed that LOV-loaded MN-SLB exhibited a sustained drug release behavior. Compared with the LOV-loaded mesoporous silica particles, LOV-loaded MN-SLB markedly suppressed the burst release. Furthermore, the pharmacokinetics and relative bioavailability of the LOV-loaded MN-SLB formulation was studied in beagle dogs after oral administration and using a commercially available immediate release formulation (Sandoz Lovastatin®) as a reference. It was found that the relative bioavailability of LOV and LOV β-hydroxy acid (LOVA) for the LOV-loaded MN-SLB formulation was 207.2% and 192.1%, respectively. In addition, MN-SLB exhibited negligible toxicity against Caco-2 and HT-29 cells in cytotoxicity assays. The results of this study indicate that the MN-SLB nanocomposite is a promising candidate as a novel oral drug delivery nanovehicle for controlling the dissolution rate and improving the oral absorption of water-insoluble drugs.

1. Introduction

The oral delivery of drugs is generally the most convenient route, as it allows painless and easy administration and, therefore, high patient compliance. However, over 70% of newly developed active pharmaceutical ingredients that are being developed today by high-throughput screening and combinatorial chemistry fall into biopharmaceutical classification system (BCS) classes II (low solubility and high permeability) and IV (low solubility and low permeability) [1,2]. Water-insoluble drugs exhibit low oral bioavailability and are ineffective therapeutically due to their incomplete absorption from the gastrointestinal tract. Hence, one of the major challenges of the pharmaceutical industry is to develop strategies to improve the aqueous solubility and dissolution rate of water-insoluble drugs [3,4]. In addition, for many hydrophobic drugs with a short elimination half-live and a low therapeutic index, it is necessary to dose at regular, frequent intervals in order to maintain the concentration within the therapeutic range. As a result, much research has been conducted into methods of improving the solubility and modulating the dissolution rate to increase the oral bioavailability of water-insoluble drugs [5–7]. In recent decades, various nanonization strategies have been proposed to increase the aqueous solubility and regulate the dissolution rate of water-insoluble drugs. These strategies include increasing the surface area

* Corresponding author at: Xuzhou Medical College, P.O. Box 62, 209 Tongshan Road, Quanshan District, Xuzhou, Jiangsu Province 221004, China.
Tel.: +86 516 83262139; fax: +86 516 83262139.
E-mail address: yanzhuozhang@126.com (Y. Zhang).

http://dx.doi.org/10.1016/j.colsurfb.2015.02.021
0927-7765/

to volume ratios of drug powders [8,9], enhancing the wettability and porosity [10,11], changing the drug crystalline state (preferably to an amorphous state) [12,13] and designing novel nanomaterials that can act as carriers for sustained release [14–17].

Ordered mesoporous silica matrices offer several advantages for drug delivery over other drug carriers, including a large inner surface area and pore volume to highly disperse the drug molecules, provide a high drug loading capacity, with tunable pore structures and excellent physicochemical stability [18,19]. In recent years, there has been considerable interest in developing ordered mesoporous silica materials as prospective oral drug carriers to improve the aqueous solubility and dissolution properties of hydrophobic drugs. The ability of ordered mesoporous silica to enhance the solubility and dissolution rate of the incorporated drug is due to their small nanopores, which can convert a crystalline drug to an amorphous form [20–22]. Furthermore, hydrophobic drugs loaded in ordered mesoporous silica can improve wettability and increase porosity [10]. However, classic mesoporous silica matrices are not suitable for sustained release of the incorporated drug molecules from the matrices following oral administration. This is mainly due to their significant burst release effect in the early stage of drug release, which may result in a higher peak concentration with the possibility of toxic effects [22–25]. In this context, how to combine the excellent properties of ordered mesoporous silica matrices with the demand for regulating drug release has attracted much attention from many researchers working on the design of controlled release drug delivery systems. Interestingly, a novel and versatile nanocarrier, the nanoporous silica particle (with a disordered, worm-like mesostructure)-supported lipid bilayer ('protocell'), which synergistically combines favorable features of both nanoporous silica particles and liposomes to address the multiple challenges of targeted delivery, has been successfully synthesized [26]. Compared with liposomes, the most extensively studied class of nanocarriers, the protocell is more stable and takes advantage of the nanoporous core to control both drug loading and release. Compared with the nanoporous silica particle, the protocell is more biocompatible and takes advantage of the lipid bilayers to reduce or eliminate the initial burst release [26,27]. However, until now, no study has explored their potential in modulating the dissolution rate and improving the bioavailability of water-insoluble drugs following oral administration.

In the current study, we have designed a novel MN-SLB nanocomposite as an oral drug carrier where monodisperse MCM (mobile crystalline material)-41-type mesoporous silica was used as the core containing a water-insoluble drug surrounded by lipid bilayers as the hydrophobic shell. To increase the storage stability of the lipid bilayers, the drug-loaded MN-SLB nanoparticles were freeze-dried. LOV (Supplementary Fig. S1) was selected as the model drug in this study. LOV is a cholesterol-lowering agent which is widely used to treat hypercholesterolemia. LOV is a lipophilic compound ($\log P = 3.91$) which belongs to BCS class II. LOV exhibits poor oral bioavailability of <5% because of its low aqueous solubility and rapid metabolism in the gut and liver [28,29]. LOV and LOV β-hydroxy acid (LOVA) have short half-lives (1–2 h), and steady-state concentrations are achieved within 2–3 days. A formulation with a high degree of oral absorption and extended delivery potential would be highly desirable for LOV [28,30]. The objective of this study was to regulate the *in vitro* dissolution and improve the *in vivo* absorption of LOV by means of MN-SLB-based nanoparticles. To achieve this goal, samples of the drug-loaded MN-SLB nanoparticles were characterized in terms of their mean particle size, morphology, specific surface area, inner structure, drug loading, physical state, solubility, dissolution rate and amorphous state stability after 6 months of storage. Furthermore, pharmacokinetic profiling of LOV and LOVA after oral administration of drug-loaded MN-SLB or the commercial immediate release tablets in fasted beagle dogs was carried out using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). In addition, the cytotoxic effects of MN-SLB on human colon carcinoma HT-29 and Caco-2 cells were also evaluated.

2. Materials and methods

2.1. Materials

Bulk crystalline LOV (USP-grade, purity ≥99%), LOVA and simvastatin (purity ≥99%) were kindly provided by Hisun Pharmaceutical Co., Ltd. (Zhejiang, China). Dipalmitoyl phosphatidylcholine (DPPC) and 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) were obtained from Avanti Polar Lipids (Alabaster, AL, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercial Sandoz Lovastatin® tablets (Sandoz, Canada) containing 20 mg of LOV were purchased commercially from a local pharmacy. Pluronic F127 was a gift from BASF (Ludwigshafen, Germany). Lactose monohydrate and pregelatinized maize starch were donated from Roquette (Jiangsu, China) and used as received. Avicel® PH microcrystalline cellulose (MCC) was supplied from FMC BioPolymer (Philadelphia, PA, USA). Purified water was obtained from a Millipore Milli-Q filtration/purification system (Billerica, MA, USA) and all other reagents were of analytical or chromatographic grade.

2.2. Synthesis of liposomes

Liposomes were prepared by the lipid film hydration and extrusion method as described previously [31]. Briefly, 16 mg DOTAP, 4 mg DPPC and 0.9 mg cholesterol were dissolved in a mixture of chloroform (18 ml) and anhydrous ethanol (2 ml) in a round-bottom flask. After the organic solvent was removed in a N1000 rotary evaporator (Eyela, Japan) under reduced pressure at 313 K, the resulting thin lipid film was further dried overnight in a vacuum chamber to ensure complete removal of the organic solvent. Then, 8 ml phosphate buffered saline (PBS, 10 mM, pH 7.4) was added to the dry lipid film which was hydrated for 1 h at 323 K with repeated vortexing. The obtained multilamellar liposomal suspension was sonicated by a JY96-IIN probe-type sonifier (Scientz, China) for 5 min and extruded four times through polycarbonate membranes with a pore size of 0.22 μm (Whatman, USA) using a mini-extruder (Avanti Polar Lipids, USA) to obtain liposomes with an average diameter of around 200 nm.

2.3. Synthesis of mesoporous nanomatrix (MN)

The ordered MN material was synthesized using *N*-cetyltrimethylammonium bromide (CTAB) as a structure directing agent and triisopropylbenzene (TIPB) as a swelling agent [32]. In a typical synthesis procedure, 0.5 g CTAB and 0.1 g TIPB were dissolved in 240 g sodium hydroxide solution (0.015 M) at 313 K in a glass container. Then, 2.5 g tetramethyl orthosilicate (TMOS) was slowly added to the surfactant solution. After 30 min stirring, the reaction mixture was kept under static conditions at the same temperature for 20 h. Then, the resulting colloidal mixture was transferred to a Teflon-coated stainless-steel autoclave and placed in an oven for hydrothermal treatment at 378 K for another 20 h. The resulting as-synthesized material was washed on a 0.2 μm membrane filter with a mixture of hydrochloric acid and ethanol (40/60) for 1 h, dried at 323 K for 1 h, and calcined in air at 773 K for 6 h to remove the surfactant template.