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Mesoporous carbon with spherical pores as a carrier for celecoxib with needle-like crystallinity: Improve dissolution rate and bioavailability

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ABSTRACT

The purposes of this investigation are to design mesoporous carbon (MC) with spherical pore channels and incorporate CEL to it for changing its needlelike crystal form and improving its dissolution and bioavailability. A series of solid-state characterization methods, such as SEM, TEM, DSC and XRD, were employed to systematically investigate the existing status of celecoxib (CEL) within the pore channels of MC. The pore size, pore volume and surface area of samples were characterized by nitrogen physical absorption. Gastric mucosa irritation test was carried out to evaluate the safety of mesoporous carbon as a drug carrier. Dissolution tests and in vivo pharmacokinetic studies were conducted to confirm the improvement in drug dissolution kinetics and oral bioavailability. Uptake experiments were conducted to investigate the mechanism of the improved oral bioavailability. The results of solid state characterization showed that MC was prepared successfully and CEL was incorporated into the mesoporous channels of the MC. The crystallinity of CEL in MC was affected by different loading methods, which involve evaporation method and melting method. The dissolution rate of CEL from MC was found to be significantly higher than that of pure CEL, which attributed to reduced crystallinity of CEL. The gastric mucosa irritation test indicated that the MC caused no harm to the stomach and produced a protective effect on the gastric mucosa. Uptake experiments indicated that MC enhanced the amount of CEL absorbed by Caco-2 cells. Moreover, oral bioavailability of CEL loaded within the MC was approximately 1.59-fold greater than that of commercial CEL. In conclusion, MC was a safe carrier to load water insoluble drug by controlling the crystallinity or crystal form with improvement in drug dissolution kinetics and oral bioavailability.

1. Introduction

Oral administration was the most commonly route of administration because of its convenience. In recent years, a large number of new chemical entities (NCE) had been developed due to advances in combinatorial chemistry and high-throughput screening technology [1,2]. However, many compounds cannot be used clinically due to their poor bio-pharmaceutical properties, such as poor aqueous solubility and low permeability through the membrane, resulting in low oral bioavailability [3,4]. As reported, the crystalline form was of particular importance to improving the dissolution kinetics of poorly water soluble drugs [5]. The dissolution kinetics could be enhanced through a number of routes, which were offered by crystal engineering using in-depth knowledge of crystallization [6,7]. In recent years, many formulations such as nanocrystalline [8–11] and microcrystal [12,13] had been applied in the pharmaceutical field owing to the advantages offered by their nanoscale size and special microcrystal characteristics.

The emergence of inorganic porous materials (such as porous silica and carbon) as drug delivery systems had opened up a new path for improving the dissolution kinetics of poorly water soluble drugs [14–16]. Compared with traditional pharmaceutical carriers, such as liposome and polymer nanoparticles, an inorganic carrier has considerable advantages in terms of particle size, shape control, stability and surface functionalization [17–19]. Inorganic material is also of great potential value because of its unique optical, magnetic, electrical and physical properties [20,21]. So mesoporous silica as a drug delivery system had been investigated to improve the dissolution kinetics of CEL in our previous study [22]. The dissolution kinetics was controlled by regulating the pore size of mesoporous silica. As far as we know, pore structure was also a significantly important property for mesoporous materials as a drug carrier. However, until now, there were rare reports about the relationship between the pore morphology and crystal form. Additionally, mesoporous carbon with extremely large specific surface area, pore volume, strong adsorption ability, high drug loading capacity and chemical inertness was very suitable as a drug delivery system for

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poorly soluble drugs [23–25]. To address this problem, we exploited MC as a carrier for CEL, in order to investigate the relationship between the spherical pore channels and the needlelike crystal form.

On the basis of our previous study, mesoporous carbon was constructed using a template of mesoporous silica with face-centered cubic pore structure. Then CEL was loaded in mesoporous carbon using different methods. Solid-state characterization methods, such as SEM, TEM, DSC and XRD, were used to systematically investigate the external morphology of the drug loading system and the relationship between the pore morphology and crystal form. The pore size, pore volume and surface area of samples were characterized by nitrogen physical absorption. Dissolution tests and in vivo pharmacokinetic studies were conducted to confirm the improvement in drug dissolution kinetics and oral bioavailability. The mechanism of the improved oral bioavailability was studied by uptake experiments. Gastric mucosa irritation test was carried out to evaluate the safety of mesoporous carbon as a drug carrier.

2. Materials and methods

2.1. Materials

Pluronic block co-polymer F127 was donated by BASF. Tetraethyl orthosilicate (TEOS), hydrofluoric acid, hydrochloric acid, sulfuric acid, sucrose and potassium chloride were purchased from Yu Wang Reagent Company (Shandong, China). 135-Trimethylbenzene (TMB) was purchased from Sigma-Aldrich (St. Louis, MO, USA). CEL (purity > 99.0%) was kindly supplied from Shenyang Funning Pharmaceutical Co., Ltd. All other chemicals used were of analytical/spectroscopic/HPLC grade. Deionized water was obtained by ion exchange. Caco-2 cells were obtained from American Type Culture Collection (ATCC). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO, USA).

2.2. Preparation and drug loading of MC

2.2.1. Preparation of MC

Mesoporous carbon was prepared using mesoporous silica were as template. The template was prepared according to the procedure reported by Zhu et al. with some modifications [22]. In the synthesis procedure of the template (mesoporous silica), firstly, F127 (template) and TEOS (silica source) formed a composite and then was homogenized by an ATS AH100D homogenizer (ATS Engineer Inc., Shanghai, China). The hydrothermal reaction formed the mesoporous silica with face-centered cubic structure. Then, the F127 was removed at 600 °C. The reaction temperature in the synthesis of mesoporous silica was 15 °C without other procedures changed.

As shown in Graphical abstract, carbon source was impregnated into the template to form the carbon–silica composite. After that, the template was removed and mesoporous carbon with spherical pore was formed. Mesoporous silica (1.0 g) was first impregnated with a solution composed of 0.95 g sucrose and 0.1 g H_2SO_4 in 2.4 g H_2O at the initial permeation. The obtained white gel was placed in an oven at 80 °C for 6 h, and subsequently, the oven temperature was increased to 160 °C at the speed of 2 °C/min for another 6 h. At the second permeation, the obtained product was heat-treated again at 80 °C and 160 °C after the addition of an aqueous solution consisting of 0.6 g sucrose, 0.06 g H_2SO_4 and 1.6 g H_2O for 6 h, respectively. The carbonization of the mixture was accomplished by heating up to 700 °C for 3 h at the speed of 3 °C/min under N_2. Finally, the carbon–silica composite was placed in 10% hydrofluoric acid at 25 °C for 48 h to remove the silica template. The final samples were filtered, washed with water, and dried at 100 °C for 24 h. The mesoporous carbon with ordered mesoporous pore structure was obtained.

2.2.2. Drug loading procedure

2.2.2.1. Evaporation method (E). Briefly, CEL ethanol solution (20 mg/mL) was mixed with MC with mass ratios of 20%, 30% and 40%. Then, the mixture was ultra-sonicated and gently stirred for 24 h at 25 °C. Finally, the solvent was allowed to evaporate under stirring and then the material was dried at 40 °C in air for 48 h. The precipitated powder was washed with ethanol. Drug-loaded samples with different mass ratios of 20%, 30% and 40% were labeled as MCE-20, MCE-30 and MCE-40, respectively.

2.2.2.2. Melting method (M). CEL (20 mg) in a glass dish was melted at about 165 °C in an oven and mixed with MC according to the mass ratio of 1:4. Then, the mixture was placed at about 165 °C again and immediately placed into the bath of liquid nitrogen. Finally, the products with a mass ratio of 20% were stored under vacuum and referred to as MCT.

As shown in Graphical abstract, the needle-like shape of CEL was changed to spherical shape after drug loading due to the restriction of pores.

2.3. Characterization techniques

2.3.1. SEM study

Morphology of the samples was evaluated using a field emission scanning electron microscope (JEOL-6700, Japan). The samples were overlaid with a thin layer of gold under vacuum before the experiment.

2.3.2. TEM study

The porous structure of the samples was characterized using TEM (Tecnai G2 20, FEI, USA). The samples were deposited on copper grids before examination.

2.3.3. XRD and DSC analysis

X-ray diffractometer (PW3040/60 Panalyii Calb.V Netherlands) was used to describe the crystalline characteristics of CEL in MC. XRD profiles were recorded from 5° to 50° with a step size of 0.02° and a scan rate of 5°/min. DSC patterns of the samples were obtained using a DSC instrument (TA Instruments, Q1000, USA). The temperature range was 50–200 °C at a heating rate of 10 °C/min.

2.3.4. Nitrogen adsorption analysis

Adsorption–desorption analysis was carried out on a surface area analyzer (SA3100, Beckman Coulter, USA). All samples (pure carrier and carrier loaded with drug) were degassed at the same temperature (e.g. 50 °C).

The surface areas were calculated according to the BET (Brunauer–Emmett–Teller) method, the pore size distributions were determined using the BJH (Barett–Joyner–Halenda) procedures and the total pore volumes were analyzed through the amount adsorbed.

2.4. Analysis of drug content

CEL was analyzed using ultraviolet (UV) spectroscopy (UV-2000, Unico, USA), while the detection wavelength was 254 nm. CEL was extracted from the samples by sonication method. The following equation was used to calculate the degree of drug loading: Drug loading (%) = $Weight_{CEL}$ / $Weight_{sample}$.

2.5. Release behavior study

Dissolution studies were performed using a USP II paddle method with a KC-8D dissolution tester (KC-8D, Tianjin Guoming Medical Equipment Co. Ltd.). The dissolution tests were carried out according to the procedure reported by Zhao et al. [27]. The dissolution medium was phosphate buffer (pH 6.8). Samples (MCT, MCE-20, MCE-30 and