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Oral bioavailability of silymarin formulated as a novel 3-day delivery system based on porous silica nanoparticles

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ABSTRACT

The purpose of this study was to develop porous silica nanoparticles (PSNs) as a carrier to improve oral bioavailability of poorly water-soluble drugs, using silymarin as a model. PSNs were synthesized by reverse microemulsion and ultrasonic corrosion methods. A 3-day release formulation consisting of a silymarin solid dispersion, a hydrophilic gel matrix and silymarin-loaded PSNs was prepared. In vitro release studies indicated that both the silymarin-loaded PSNs and the 3-day release formulation showed a typical sustained-release pattern over a long period, about 72 h. The in vivo studies revealed that the 3-day release formulation gave a significantly higher plasma concentration and larger area under the concentration–time curves than commercial tablets when orally administered to beagle dogs. This implies that the prepared 3-day release formulation significantly enhanced the oral bioavailability of silymarin, suggesting that PSNs can be used as promising drug carriers for oral sustained release systems. Thus providing a technically feasible approach for improving the oral bioavailability and long-term efficacy of poorly soluble drugs.

1. Introduction

Silymarin, an antihepatotoxic polyphenolic substance extracted from fruit seeds of the milk thistle plant (*Silybum marianum* Gaertn), has been widely used to treat a variety of liver disorders including acute and chronic viral hepatitis [1–3], alcoholic liver diseases [1], toxin- and drug-induced hepatitis and cirrhosis, fatty liver radiation, and toxicity. It is mainly composed of four flavonolignans, silybin, isosilybin, silydianin and silychristin, of which silybin is the most biologically active component, representing approximately 60–70% [3–5]. However, the therapeutic effects of silymarin are restricted due to its poor water solubility, resulting in poor oral absorption and low bioavailability after oral administration [6–8]. Xiao and co-workers reported that after oral administration of pure silybin, it was not detected in plasma [9]. In addition, Wu and co-workers reported that absolute oral bioavailability of silybin in rats was approximately 0.95% due to the poor solubility and extensive pre-systemic metabolism of the drug [10]. It was also reported that only 20–50% of silymarin was absorbed from the gastrointestinal tract after oral administration [6]. Furthermore, silymarin's poor water solubility and poor absorption may be attributed to its poor permeation across intestinal epithelial cells [11,12]. Therefore, developing strategies to overcome these difficulties and to enhance the oral bioavailability of silymarin are highly desirable.

In recent times, different strategies have been investigated to improve the dissolution and bioavailability of silymarin. These strategies included silymarin/polyvinylpyrrolidone solid dispersion (SD) pellets [13], a silymarin or dehydrosilymarin proliposome [9,14,15], a silymarin self-microemulsifying drug delivery system (SMEDDS) [16], a silybin–phospholipid complex [17], silybin-loaded povidone–sodium cholate–phospholipid mixed micelles [18] and silymarin liposomes [19]. Again, Xiao and co-workers reported that a silymarin proliposome improved the oral bioavailability of silymarin in beagle dogs and enhanced gastrointestinal absorption. The relative bioavailability of silymarin SMEDDS is superior to that of silymarin PEG 400 solution and its suspension. Although the oral administration of these drug delivery systems in previous studies showed improved bioavailability, only few of these formulations that displayed sustained release of silymarin for more than 16 h in vivo have been reported. Thus improvement in bioavailability has been limited.

Over the past few decades, the application of mesoporous silica nanoparticles (MSNs) as a drug delivery system has attracted considerable attention [20–22]. It was reported that ordered mesoporous silica materials could be developed into a broad-spectrum

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formulation platform for poorly soluble drugs [20]. MSNs possess many unique features such as excellent biocompatibility and biodegradability, a large specific surface area and pore volume, tunable pore size with a narrow pore size distribution and excellent physicochemical stability. The porous structures with hundreds of empty channels (mesopores) which can also be modified easily are able to absorb/encapsulate relatively large amounts of drug molecules, thus allowing the control of drug release with high precision. Ukmar and co-workers found out that the controlled release of drugs from ordered porous materials had a close relationship with the pore size and drug molecule–wall attractions [23–25]. Drug–wall interactions can be achieved via the surface modification of the drug-delivery materials [23,26]. A number of studies have reported the sustained release of drug-loaded porous silica nanoparticles (PSNs) in vitro. Li and co-workers reported the typical sustained release of Brilliant Blue F-loaded porous hollow silica nanoparticles (PHSNPs) in vitro, from which drug dissolution was observed for as long as 1140 min [27]. In addition, Chen and co-workers found out that the release of cefradine from PHSNP followed a three-stage pattern lasting for 12 h [28] and was due to the drug release from the surface pore channels in the wall and the inner hollow part of PHSNPs. Safety has also been investigated with an in vivo study showing that MSNs exhibited no visible cytotoxicity against LX-2 cells over a broad spectrum of concentrations and possessed good blood compatibility [29]. Furthermore, Bimbo and co-workers reported that 80% of PSNs were dissolved in biorelevant media after 144 h [30]. They also found that the degradable product was harmless to Caco-2 human epithelial colorectal adenocarcinoma cells [31]. In all, the PSNs exhibited excellent biocompatibility, biodegradation, in vivo stability, low cytotoxicity and nonimmunogenic profiles [31], thereby making these nanoparticles an ideal candidate for oral drug delivery. However, very few studies have focused on the in vivo pharmacokinetics and bioavailability of drug-loaded PSNs, which is of great importance for the evaluation of in vitro and in vivo correlations. It is the aim of this study to develop an approach for in vivo evaluation of drug-loaded PSNs for better investigation of the efficacy of prepared formulations.

Improving the solubility of poorly soluble drugs and preparing sustained-release formulations have always been a great challenge to researchers. Poorly soluble drugs are solubilized prior to being developed into sustained-release formulations that can typically release for 12 h or at most 24 h, with one or two administrations per day. With regard to the promising properties of PSNs, this study was targeted at developing PSNs as carriers of the poorly soluble drug silymarin in order to improve its bioavailability. This obviously provided the basis for the development of a new long-acting sustained-release formulation for poorly soluble drugs. The study employed a reverse microemulsion method for the synthesis of monodispersed nonporous silica nanoparticles to form "cores" and an ultrasonic corrosion method to create regular nanometer-sized pores with sodium carbonate (Na_2CO_3) solution. The pharmacokinetics of drug-loaded PSNs and their formulation was investigated in beagle dogs and the oral bioavailability was compared with that of Legalon®, a commercial product.

2. Materials and methods

2.1. Materials

Silymarin was kindly supplied by Jiangsu Zhongxing Pharmaceutical Co., Ltd. (Zhenjiang, PR China). Commercial silymarin tablets (Legalon®, MADAUS GmbH, Germany) were used as a reference product. Tetraethoxysilane (TEOS), hydroxypropyl methylcellulose (HPMC K4M) and low-substituted hydroxypropyl cellulose (L-HPC) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, PR China). Lecithin (Leci) and Eudragit E100 were purchased from Shanghai Chineway Pharmaceutical Technical Company, Ltd. (Shanghai, PR China). Octylphenol polyoxyethylene (NP-10) was provided by Shanghai Junhui Chemical Factory (Shanghai, PR China), cyclohexane (CHX) by Shanghai Chemical Reagent (Shanghai, PR China), α-naphthol by Tingxin Chemical Reagent Factory (Shanghai, PR China), polyvinylpyrrolidone K30 (PVP K30) by Shanghai Sunpower Chemical Co., Ltd. (Shanghai, PR China), Na_2CO_3 by Shanghai Hongguang Chemical Factory (Shanghai, PR China) and dialysis bags by Shanghai Yuanju Biologic Technology Co., Ltd. (Shanghai, PR China). All other chemicals and solvents were of analytical grade. Double-distilled water was freshly prepared in the laboratory.

2.2. Synthesis of solid nanoparticles (SNs)

The SNs were synthesized by the reverse microemulsion method. NP-10, butanol and 2 ml ammonia (25.6%, w/v) were mixed with 50 ml CHX, and agitated for 15 min to obtain the reversed emulsion. The TEOS was slowly added to the system with stirring for 24 h. An equal volume of alcohol was also added to the emulsion, which was then sonicated at 300 W for 30 min. The resulting nanoparticle suspension was centrifuged at 15000 *g* for 15 min at 4 °C using an ultracentrifuge (Heraeus Biofuge, Stratos, Germany). The pellet was washed three times with ethanol followed by double-distilled water successively. Finally, a small amount of double-distilled water was added to the resulting product, which was then lyophilized to obtain SNs.

2.3. Synthesis of porous silica nanoarticles

The PSNs were prepared by the ultrasonic corrosion method. The SNs (100 mg) were added to 100 ml of 0.6 M Na_2CO_3 solution and the resulting suspension sonicated at 200 W at 65 °C. It was then centrifuged at 15,000 *g* for 15 min at 4 °C. The precipitate was washed with double-distilled water several times and lyophilized to obtain PSNs.

According to the previous studies, temperature (A), ultrasonic time (B) and ultrasonic power (C) had the most significant effects on the properties of prepared PSNs [32]. The optimum preparation conditions were obtained by orthogonal experimental design and single factor experimentation, thus arranging the three factors above with four levels for each factor. Sixteen different sets of experiments were performed under conditions of different parameter combinations according to the standard L_{16} (4^5) table as shown in Table 1. Table 2 shows the variance analysis of three factors on PSNs.

2.4. Drug impregnation in PSNs and SNs

The PSNs (2 g) were immersed in an ethanol solution of silymarin at a concentration of 0.2 g ml^{-1} and stirred for 24 h. The resulting suspension was purified by centrifugation. After the removal of the supernatants, 20 ml ethanol was added to the nanoparticles and the solution was thoroughly mixed on a vortex mixer for 1 min. It was then centrifuged at 10,000 *g* for 5 min with a Mini Sin centrifuge (Eppendorf, Germany). This step was repeated three times to remove silymarin adsorbed on the surface of PSNs. The silymarin concentration in the ethanol solution was then determined with a UV-Vis spectrophotometer (UV-2401PC, Shimadzu). The resulting product was resuspended in double-distilled water and freeze-dried to obtain silymarin-loaded PSNs. SNs were also taken through the same procedure to obtain silymarin-loaded SNs.