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Reduced Burst Release and Enhanced Oral Bioavailability in Shikimic Acid—Loaded Polylactic Acid Submicron Particles by Coaxial Electrospray

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ABSTRACT

In this study, using the coaxial electrospray method, we prepared submicron particles of the water-soluble drug shikimic acid (SA) with polylactic acid (PLA) as a polymer, to reduce the burst release and enhance the oral bioavailability. *In vitro* release study performed in HCl solution (pH 1.2) showed that the coaxial electrospray submicron particles could reduce burst release effect and presented a sustained release profile, compared with free SA and the particles prepared by electrospray method. The absorption of SA in the intestinal tract, studied using an *in situ* perfusion method in rats, also revealed jejunum as the main absorptive segment followed by duodenum and ileum. Moreover, the SA-loaded particles greatly enhanced the absorption of SA in the tested intestinal segments. The intestinal absorption rate was not enhanced with increasing drug concentration (5-15 μg/mL) which suggested that active transport or facilitated diffusion could play vital role in SA absorption. In addition, the SA-loaded PLA coaxial electrospray particle exhibited a prolonged plasma circulation with enhanced bioavailability after oral administration. In all, the coaxial electrospray technique could provide notable advantages for the oral delivery of SA, thereby enhancing its clinical application.

Introduction

Shikimic acid [SA, 3R, 4S, 5R-(−)-3, 4, 5trihydroxy-1-cyclohexene-1-carboxylic acid] was originally isolated in 1885 by Eykman from the fruit of *Illicium religiosum* Sieb.et Zucc. (known as Chinese star anise).[1] After several decades since its appearance, SA has been shown to occupy a major position in market as an indispensable starting material for the synthesis of the antiviral drug, Oseltamivir (Tamiflu).[2] In certain microorganisms, SA was presented as a metabolic precursor for the biosynthesis of aromatic compounds, such as amino acids, phenylalanine and tyrosine, indole derivatives, alkaloids, tannins, flavonoids, and lignin.[2-4] Although SA was confirmed to be toxic and carcinogenic in rats,[5] its derivatives, triacetylshikimic acid and isopropylidene shikimic acid, possessed anti-inflammatory properties as they could inhibit COX-1 and COX-2 activities and also decrease platelet aggregation and blood clot formation.[6,7] Besides, SA has been associated with multiple biological properties including antioxidant, antibacterial, and analgesic activities.[8,9] Moreover, Ma et al.[10] also proved that the SA could prevent focal cerebral ischemic injury after middle cerebral artery thrombosis in rat. Altogether, these reports demonstrate that SA is a promising therapeutic agent for cardiovascular, peripheral, and cerebral vascular diseases and could be ideal for the treatment of acute coronary syndrome.

Currently, several researchers are active in the study of natural, biotechnological, and synthetic sources as well as pharmacological applications of SA.[11-13] However, at the molecular level, SA studies on biological activities or preparation are very limited. Until now, to the best of our knowledge, no research has been reported on the *in vitro* analysis and *in vivo* bioavailability.

Nowadays, numerous efforts have been made to improve the bioavailability and solubility for poorly water soluble drugs, such as micelle, microemulsion, and microparticles.[14-16] However, recent advances in nanotechnology (liposomes, nanocapsules, nanoparticles, microspheres, carbon nanotubes, and so forth) have

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shown promise for water-soluble drug in controlled drug release resulting in increased drug absorption. Such nanotechnology has also been useful in maintaining steady therapeutic levels of drugs over an extended period compared with traditional drug preparations.[17-21] Among these nanocarriers, nanoparticles based on electrospray technique have emerged as one of the most useful modalities for suitable drug carriers. Electrospray is a simple and versatile procedure for the fabrication of particles with diameters varying from submicron to micron.[22] It forms nanoparticles by vaporizing the solvent. The whole process is conducted with high voltage from a metal capillary tip to induce a charge on the surface of liquid, thus leading to the formation of nanoparticles of different sizes. In addition, a polymer solution could produce nonwoven fibrous mats with desired size range by controlling factors such as the solution and process parameters including applied voltages, collecting distances, and solution flow rates.[23]

In the nanoparticle formulation based on electrospray technique, particular interest has been focused on the use of polyester materials such as polylactic acid (PLA). PLA, one of the important candidates for nanoporous fibers fabricated for biomedical application, has been extensively used as microparticle carrier in drug delivery systems for many bioactive molecules owing to its nontoxic, excellent biocompatible and biodegradable properties.[24] Bognitizki et al.[25] was the first to fabricate nanostructured electrospun PLA fibers using dichloromethane solvent. It was reported that the incorporation of the inorganic particulate in the polymer matrix could change morphological structure of the nanofiber or nanoparticle.[26] However, “initial burst” release, the phenomenon whereby a large part of the encapsulated drug is released in a short time just after administration, is one of the major disadvantages associated with the nanoparticles.[27] Ibrahim et al.[28] reported that the initial burst release of insulin led to an early loss of 66% of the protein dose from PLA microsphere. To overcome this limitation, coaxial electrospray method regarded as one of the most significant breakthroughs in this area opened a new way for generating nanoparticles from polymer solutions by manipulating 2 different liquids using a concentric spinneret.[29] This specific electrospray technique uses the spray head assembled by axially inserting an inner capillary in an outer tube. Dual-capillary electrospray has been demonstrated to directly produce monodisperse droplets with the outer liquid encapsulating the inner one.[30] Moreover, the separate liquid in different capillary offers great flexibility to encapsulate drugs of various types, such as proteins, enzymes, and antibiotics, without losing their bioactivity. The coaxial process has gradually been demonstrated to be a useful tool in preventing clogging of spinneret for continuous preparation of pure polymer nanoparticles such as polyvinylpyrrolidone and polyacrylonitrile.[31,32] Moreover, this system could show high drug encapsulation efficiency and mitigate the initial burst release because the drug is fully encapsulated in the core of particles.[33]

Till date, neither release behavior *in vitro* nor bioavailability *in vivo* of SA has been investigated. Hence, for the first time, phospholipid-coated SA-loaded PLA submicron particles based on coaxial electrospray method was successfully prepared to evaluate its release characteristics and bioavailability as against the free SA.

Materials and Methods

Materials

SA of purity 99% was supplied by Energy Chemical Co., Ltd. (Shanghai, China). PLA (pharmaceutical grade, molecular weight of 10,000) was produced by Jinan Daigang Biomaterial Co., Ltd. (Shandong, China). Phospholipid (soybean lecithin, analytical injection grade, with phosphatidyl-choline content of 70%) was purchased from Taiwei Pharmaceutical Co., Ltd. (Shanghai, China). Acetone of analytical grade was provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The filter membrane and cellulose nitrate membrane were supplied by Xingya Purifying material company (Shanghai, China). Pure chromatographic methanol was obtained from Hanbon Sci. & Tech. (Jiangsu, China). Chromatographic grade acetonitrile was purchased from Honeywell Burdick & Jackson (Muskegon, MI). Analytical pure ethyl acetate was purchased from Kelong Chemical Reagent Factory (Sichuan, China). Double-distilled water was produced by the machine equipped with a millipore water purification system (Millipore Corporation, Billerica, MA). All the other chemicals used in the study were of analytical grade and obtained commercially.

Fabrication of SA-Loaded Electrospray Submicron Particles

The electrospray process was performed using a high-voltage DC power supply (HB-Z303-1AC; Heng Bo High Voltage Power Supply Plant, Tianjin, China) at 18 KV, an automatic syringe pump (LSP01-1A; Baoding Longer Precision Pump Co., Ltd, Hebei, China) with a constant speed of 0.3 mL/h, a syringe equipped with a stainless steel needle (inner diameter, 0.5mm) with the tip-to-collector distance fixed at 15cm. The polymer solution was prepared by mixing PLA and SA (at a weight ratio of 3:1) in methanol and acetone (at a volume ratio of 1:4), making the final concentration of SA 0.4% (w/v). Afterward, the SA-loaded electrospray particle (F-ES) was obtained on the surface of aluminum foil.[34]

Fabrication of SA-Loaded Coaxial-Electrospray Submicron Particles

The core electrospinnable SA solution was prepared as mentioned in the previous section with the sheath solution consisting of 50-mg phospholipid in 5-mL methanol.

The coaxial electrospray process was operated using 2 syringe pumps and a high-voltage power supply (Fig. 1). A homemade coaxial electrospinneret was used to conduct the coaxial electrospray. After optimization from previous experiment, the flow rate of sheath solvent and core solution set at 0.3 mL/h with a higher voltage (21 KV) compared to previous electrospray processes (18 KV) proved to be suitable. All electrospray procedures were carried out under ambient conditions to form the SA-loaded coaxial electrospray submicron particles (F-COES).

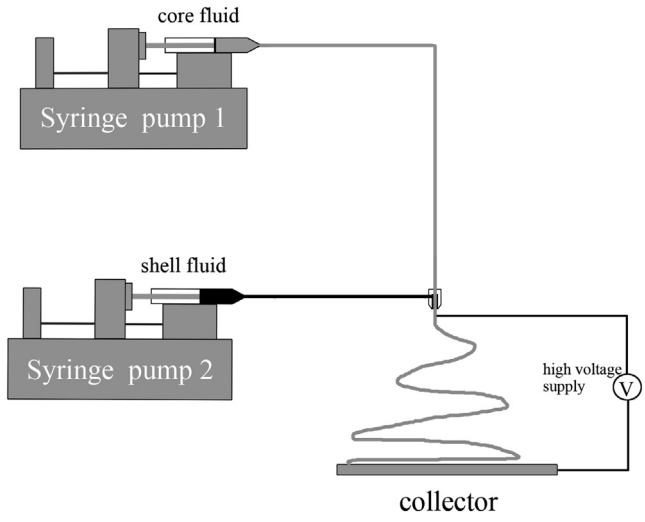

Figure 1. A schematic diagram of coaxial electrospray setup.