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Research Article

Preparation and Physicochemical and Pharmacokinetic Characterization of Ginkgo Lactone Nanosuspensions for Antiplatelet Aggregation

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ABSTRACT

The aim of this study was to investigate the potential of nanosuspensions (NSs) in improving the dissolution and absorption of poorly water-soluble ginkgo lactones (GLs), including ginkgolide A, ginkgolide B, and ginkgolide C. Liquid GL-NSs were prepared by a combined bottom-up and top-down approach with response surface methodology design, followed by freeze-drying solidification. Physicochemical characterization of the prepared freeze-dried GL-NSs was performed by photon correlation spectroscopy, scanning electron microscopy, powder X-ray diffraction, and differential scanning calorimetry. *In vitro* dissolution and *in vivo* bioavailability of ginkgolide A, ginkgolide B, and ginkgolide C in freeze-dried GL-NSs were evaluated with GLs coarse powder as control. Their inhibitory effects on platelet aggregation were also comparatively analyzed. GLs existed in an amorphous state in the prepared freeze-dried GL-NSs. The particle size, polydispersity index, zeta potential, and redispersibility index of freeze-dried GL-NSs were around 286 nm, 0.26, −25.19 mV, and 112%, respectively. The particle size reduction resulted in much more rapid and complete dissolution of ginkgolides from GL-NSs than coarse powder. Comparison with GLs coarse powder, freeze-dried GL-NSs showed a significant decreased T_{max}, 2-fold higher peak concentration, and 2-fold higher area under plasma concentrations curve for 3 ginkgolides and exhibited significantly higher antiplatelet aggregation effect.

Introduction

Ginkgo lactones (GLs), including ginkgolide A (GA, PubChem CID: 6419993), ginkgolide B (GB, PubChem CID: 6324617), and ginkgolide C (GC, PubChem CID: 441295), are a unique group of natural diterpenoide lactones from the leaves of the *Ginkgo biloba* tree.[1] These natural lactones could inhibit the platelet-activating factor (PAF) by binding to its membrane receptor and then producing anticoagulant effect. GLs have long been used to treat diseases of the central nervous system, such as degenerative dementia and neurosensory disorders.[2] It was reported that GB could improve memory and cognition,[3,4] which may be due to its ability to scavenge free radicals and inhibit seryl and aspartyl proteases and, hence, protect against neural damage.[5] In Europe and the United States, products containing extracts of GLs are top sellers in the growing market of herbal medicines.[6] Nevertheless, GLs are poorly water-soluble compounds with low oral bioavailability, which greatly limits their formulation, development, and clinical application.[7,8]

For such hydrophobic compounds, poor solubility would result in a slow dissolution and may create delivery problems such as low oral bioavailability and erratic absorption. So far, several formulation strategies for GLs have been investigated, including liposomes,[9] solid dispersion,[10] and self-emulsifying drug delivery system.[11] Although solubility and dissolution of GLs have been significantly enhanced by these formulation techniques, drug-loading capacity and encapsulation efficiency were not sufficient especially for GLs with high clinical doses.

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Nanosuspensions (NSs) are carrier-free colloidal drug-delivery systems that contain drug particles and minimal stabilizers. The mean sizes of these drug particles are in the nanometer range, typically between 10 and 1000 nm. Nano-sized particles could significantly enhance solubility and dissolution rate of the drug. In addition, mucosal adhesive property of nano-sized particles prolongs the contact time of drugs to gastrointestinal tract epithelium. These factors could be involved in the oral bioavailability enhancement of poorly soluble drugs.[12-14]

NS can be prepared using 2 typical approaches: top-down and bottom-up technologies. Top-down processes reduce the size of large drug particles using wet-milling techniques, such as high-pressure homogenization (HPH). The mean particle size of NS prepared by HPH is usually between 400 nm and 1000 nm.[15,16] However, HPH is a high energy–consuming and low-efficiency approach.[17] It generates considerable heat so that it is inadequate for processing thermolabile materials. In bottom-up processes, the drug is dissolved in an organic solvent and then precipitated with an antisolvent in the presence of a stabilizer.[18,19] This method usually makes NS formulation unstable through the generation of various unstable polymorphs, hydrates, or solvates. Needle-shaped particles usually formed owing to rapid growth in 1 direction, which influences the physical stability of the NS.[17]

Therefore, a combined bottom-up and top-down approach was studied to prepare an NS having narrow size distribution.[20] During the physical modification (bottom up) process, first, the drugs and stabilizers were initially dissolved in different, suitable solvents. Second, the particle size was refined during crystallization using an antisolvent method, and a hydrophilic drug/stabilizer matrix (modified drug) was obtained. Third, HPH (a top-down process) was used to prepare an NS with a particle size <300 nm. At last, the NS was solidified by freeze drying or spray drying.

Response surface methodology (RSM), including Box-Behnken design (BBD), central composite design, and 3-level full factorial design, is one of the experimental design methods that can be used to optimize processes and devices. Although many factors and interactions affect the desired response, it is an effective tool.[21] RSM is a statistical technique of optimizing complex preparation procedures. Through establishing a less laborious and less time-consuming mathematical model, the quantitative data from appropriate experimental design can be used to evaluate multiple parameters, as well as their interactions.[22,23] Compared with other RSM, the BBD is more efficient than 3-level full factorial designs and the central composite design.[24]

In the present study, modified high-pressure homogenization (MHPH) was used to prepare liquid NS of GLs (GL-NS) using particle size and polydispersity index (PI) as responses of RSM followed by freeze-drying solidification. Physicochemical characterizations of the prepared NS were carried out by photon correlation spectroscopy (PCS) using a Zetasizer (3000SH, Malvern Instruments Ltd., UK), scanning electron microscopy (SEM), powder X-ray diffraction, differential scanning calorimetry (DSC), and *in vitro* dissolution. In addition, pharmacokinetic study of individual GL (i.e., GA, GB, and GC) in GL-NSs were also investigated and compared with that in GLs coarse suspensions (GL-CSs). Moreover, comparisons of antiplatelet aggregation effect were made after oral administration of GL-NSs and GL-CSs, respectively.

Materials and Methods

Materials

GA, GB, GC, and ketoprofen (internal standard) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purities of the previously mentioned ingredients were >98%. Poloxamer 188 (P-188) and hydroxypropyl methyl cellulose (HPMC) were provided by BASF (Ludwigshafen, Germany). Methanol (HPLC-grade) was purchased from Merck (Darmstadt, Germany). Ethyl acetate, ammonium acetate, and other chemical reagents of analytic grade or better were obtained from Sinopharm Chemical Reagent Co. (Nanjing, China). The standardized GLs extract product containing GA (24%), GB (23%), and GC (48%) was provided by our laboratory.

Animals

Ninety-two Sprague Dawley male rats weighing 240 ± 20 g were supplied by the Animal Center of Nanjing Medical University (Nanjing, China). They were housed in controlled environmental conditions at 25°C ± 2°C and 50% ± 10% relative humidity under a 12-h dark-light cycle. The rats were kept with free access to food and water until 12 h before experiments. Animal welfare and experimental procedures were performed strictly in accordance with the *Guide for the Care and Use of Laboratory Animals* and the ethics regulations of Nanjing University of Chinese Medicine.

Experimental Design

RSM was used to investigate the influence of variables, namely the effect of the P-188/HPMC ratio, final homogenization pressures, and cycle numbers on the particle size and PI of the liquid GL-NSs. A BBD, with 3 factors each varied at 3 levels and with 3 center-point replications as well as with a second-order response surface, was used for the experimental design. The homogenization pressures (A), P-188/HPMC ratio (B), and cycle numbers (C) were used as 3 independent variables, whereas the particle size (R_1) and PI (R_2) were dependent variables. Table 1 shows the factors and the levels at which the experiments were carried out. All trials were carried out in triplicate. The experimental results obtained are expressed as mean ± SD.

Preparation of GL-NSs

GLs powder (600 mg) was dissolved in 10-mL of ethanol and then added drop-by-drop to 100-mL solution containing 5% P-188 and 5% HPMC(wt/vol) with a stirring speed of 600 rpm for 2 h

Table 1
Box-Behnken Design for Optimization of P-188/HPMC Ratio, Homogenization Pressures, and Cycle Number

Runs	Homogenization Pressures (psi), (A)	P-188/HPMC Ratio (B)	Cycle Number (C)
1	0 (14,500)	1 (1.5)	−1 (8)
2	0 (14,500)	0 (1)	0 (10)
3	0 (14,500)	1 (1.5)	1 (12)
4	0 (14,500)	0 (1)	0 (10)
5	1 (17,400)	0 (1)	−1 (8)
6	−1 (11,600)	−1 (0.5)	0 (10)
7	−1 (11,600)	1 (1.5)	0 (10)
8	0 (14,500)	−1 (0.5)	−1 (8)
9	1 (17,400)	−1 (0.5)	0 (10)
10	1 (17,400)	0 (1)	1 (12)
11	−1 (11,600)	0 (1)	−1 (8)
12	0 (14,500)	−1 (0.5)	1 (12)
13	0 (14,500)	0 (1)	0 (10)
14	0 (14,500)	0 (1)	0 (10)
15	0 (14,500)	0 (1)	0 (10)
16	1 (17,400)	1 (1.5)	0 (10)
17	−1 (11,600)	0 (1)	1 (12)