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Preparation and Evaluation of Glaucocalyxin A Sustained-Release Pellets Based on Phospholipid Complex System with Enhanced Bioavailability

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Keywords: Glaucocalyxin A; Sustained release pellet; Phospholipid complex; Bioavailability; Solubility

Abstract

Objective: Glaucocalyxin A (GLA) suffers from low oral bioavailability and rapid in vivo metabolism. Therefore, the purpose of this study was to develop a new formulation to enhance the oral bioavailability simultaneously sustained release of GLA.

Material and methods: GLA-phospholipid complex was firstly formulated by solvent-evaporation method to improve the solubility of GLA. Differential scanning calorimetry, powder X-ray diffraction, scanning electron microscopy, and solubility study were used to characterize the GLA-phospholipid complex. And then, the optimized GLA-phospholipid complex was selected to prepare GLA-phospholipid complex sustained release pellets by extrusion-spheronization and fluidized bed coating technology. The prepared pellets were studied by in vitro drug release study and administered to beagle dogs to evaluate the oral bioavailability of GLA-phospholipid complex and GLA-phospholipid complex sustained release pellets.

Results: The results illustrated that GLA in GLA-phospholipid complex was either molecularly dispersed or in an amorphous form with 13.8-fold water solubility than the free GLA. The pharmacokinetic studies in beagle dogs demonstrated that GLA-phospholipid complex and GLA-phospholipid complex sustained-release pellets showed 2.19-fold and 2.07-fold improvement than the free GLA by oral dosage, respectively. Further, steady plasma concentration, and prolonger Tmax were simultaneously obtained from GLA-phospholipid complex sustained release pellet.

Conclusion: These outcomes suggested that the combination of phospholipid complex and sustained release pellets could enhance the oral bioavailability and prolong the action time in vivo which provided a promising delivery system for GLA.

Abbreviations

GLA: Glaucocalyxin A; GLA-PLC 1: GLA-Phospholipid Complex; DSC: Differential Scanning Calorimetry; PXRD: Powder X-ray Diffraction; SEM: Scanning Electron Microscopy; GLA-PPM: GLA-Phospholipid Physical Mixture

Introduction

In the last two decades, about 40% of discovered active pharmaceutical ingredients either from the synthetic or natural origin suffers from low oral bioavailability [1-3]. This might be attributed to the low water solubility and/or permeability of drug across the biological membrane [4,5]. Glaucocalyxin A (GLA) is a biologically active *ent*-kaurane diterpenoid isolated from *inulajaponica var*, a traditional Chinese medicinal herb that grows in the northeastern of China [6,7]. The structure of GLA is shown in with molecular formula of C20H28O4 (Figure 1). GLA has been widely used in China folk medicine as cytotoxicity and antitumor, anti-bacterial, anti-inflammatory, stomachic and anthelmintic agent for more than 30 years [8].

However, there are two main disadvantages of GLA for its practical use as a therapeutic agent, one is the poor water solubility caused low oral bioavailability, the other is the rapid *in vivo* metabolism which need the patient to take the medicine more than three times a day [9,10]. To overcome the drawbacks, several strategies had been proposed to increase the water solubility of GLA including nanosuspensions [11], nanoparticle [12], inclusion complex [13], and self-nanoemulsion [14] etc.

Those researches demonstrated that when the GLA formulation with a better solubility, GLA showed stronger antitumor activity compared with the free drug [11,12]. Nevertheless, there was few research focus on extending the action time of medicament within human body along with improving the oral bioavailability of GLA. As a result, there is an urgent demand to develop a new formulation with prolonged actions and enhanced oral bioavailability of GLA.

In recent years, the technique of lipid-based drug delivery

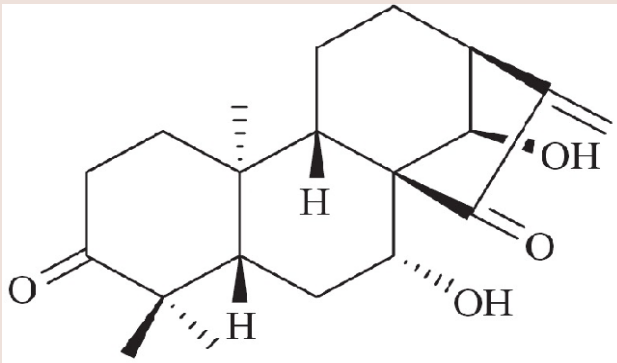

Figure 1: Chemical structure of GLA.

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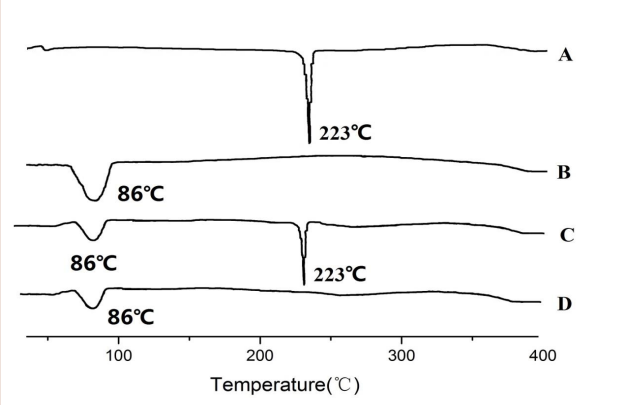

Figure 2: DSC thermographs of GLA (A), phospholipid (B), GLA-PPM (C), and GLA-PLC (D).

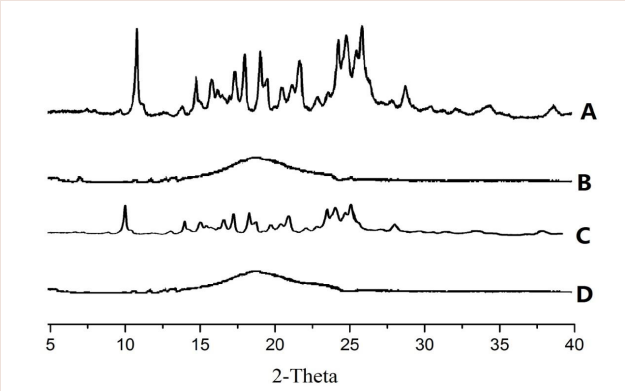

Figure 3: PXRD patterns of GLA (A), phospholipid (B), GLA-PPM (C), and GLA-PLC (D).

Table 1: Orthogonal factor level table.

Level	Factor			
	A Temperature (℃)	B Time (h)	C Concentration (mg/l)	D Drug: hospholipid (mol/ol)
1	30	1.5	5	1:0.5
2	40	2	10	1:1
3	50	3	15	1:2

system, such as liposomes [15,16], solid lipid nanoparticles [17,18], phytosomes etc. [19]. Have acquired more attention as they can resolve major drawbacks related to drug delivery. However, there are some shortcomings, like drug leakage, low drug loading, and poor stability that remain to be a major concern for liposome. Similarly, low inherent incorporation rate, high tendency for drug expulsion, and unpredictable gelation tendency of solid lipid nanoparticles restrict their overall utilization [20,21]. Besides that, the related manufacturing process is sophisticated and difficult to scale up. Among these, the drug-phospholipid complex has become one of the most successful strategies for improving the oral bioavailability of a number of poorly water soluble drugs by enhancing solubility, permeability, and by improving metabolic stability in gastrointestinal tract [22-24]. Being comprised with such property, phospholipid complex is a suitable delivery vehicle for drugs which have limited solubility (BCS class II) limited permeability (BCS class III), and both (BCS class IV). Meanwhile, it is relatively cheap, easy to formulate, and easy to scale up compared with other lipid-based drug delivery systems [25].

Spherical free-flowing pellets, as multiple unit sustained release drug reservoirs of narrow size distribution are popular in commercial products. The pellets offer many clinical advantages compared with single unit dosage forms, such as less variability release profiles [26], stable plasma concentrations [27], reduced intra-and inter-subject variability on drug plasma [28], decreased local irritations, less dose dumping risk and great reproducibility [29].

Therefore, in this study, in order to enhance the oral bioavailability and prolong the action time of GLA *in vivo*, we developed a novel GLA-Phospholipid Complex (GLA-PLC) sustained release pellets formulation to take advantage of the solubility and absorption enhancing effect of phospholipid complex and release rate-controlling capacity of the sustained release pellets.

Materials and Methods

Materials

Glaucocalyxin A was obtained from College of Pharmaceutical Science, Soochow University (Suzhou, China). Lipoid E80 (trade name of phospholipid)was purchased from Shanghai Dongshang BiologyTechnique Ltd. (Shanghai, China). Lactosewas purchased from Meggle. (Germany). Kollicoat SR 30 D was purchased from BASF (Germany). Unless otherwise stated, all other materials were of analytical grade.

Preparation of GLA-PLC

Solvent evaporation method was applied to prepare GLA-PLC. In short, GLA-PLC was prepared with drug and phospholipid in 1:0.5, 1:1, 1:2, or 1:3 molar ratios. Accurate quantity of GLA and phospholipid were taken in a 250 mL round bottom flask and dissolved in anhydrous ethanol to obtain uniform solution with the drug concentration of 2 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml, or 20 mg/ml. The prepared mixture solution was refluxed at 25 ^{0}C, 30 ^{0}C, 40 ^{0}C, 50 ^{0}C or 60 ^{0}C for 0.5 h, 1 h, 1.5 h, 2 h, 3 h or 4 hunder constant stirring at 100 rpm to form GLA-PLC. Subsequently, anhydrous ethanol was evaporated by rotary evaporation under reduced pressure to give solid product.

Based on the single factor experiment, GLA-PLC preparation

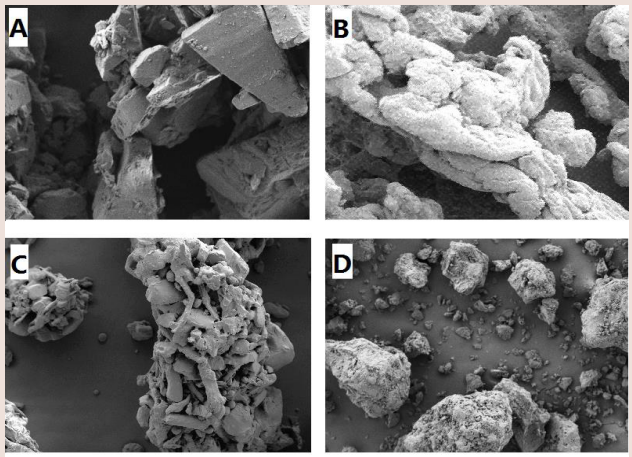

Figure 4: SEM photomicrographs of GLA (A), phospholipid (B), GLA-PPM (C), and GLA-PLC (D).

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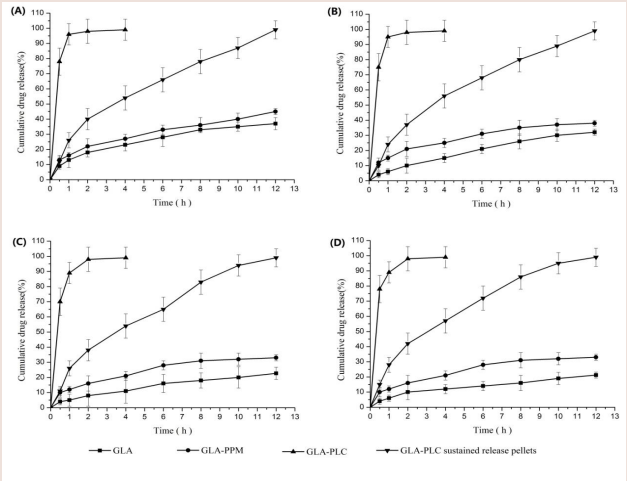

Figure 5: Cumulative drug release of GLA from pH 1.2 (A), pH 4.5 (B), pH 6.8 (C) and pH 7.4 (D) dissolution medium. Data are expressed as mean ± SD. (n = 3).

conditions were optimized by L_9 (3^4) orthogonal experiment, which can be found in Table 1. The dried residues were placed in a desiccator in room temperature until used.

The same ratio of GLA and phospholipid was simply mixed to form GLA-phospholipid physical mixture (GLA-PPM). The resulting samples was stored in a desiccator in room temperature until used.

The combination ratio of GLA-PLC

The combination ratio of GLA to phospholipid was measured under the difference of solubility between GLA and GLA-PLC [30]. GLA-PLC could be dissolved in *n*-hexane, but free GLA could not be dissolved in *n*-hexane. The combination ratio was calculated on the following basic equation (Eq. (1):

$$\text{Combination ratio} = \frac{m_1}{m_2} \times 100\% \quad (1)$$

Where, m1 is the combination amount of GLA in the GLA-PLC, and m2 is the total amount of GLA in the GLA-PLC.

Characterization of GLA-PLC

Determination of GLA content in phospholipid complex: The content of GLA in the complex was determined by reversed-phase HPLC method (Agilent 1260, Agilent Technologies, Inc., USA) using a Phenomenex Luna C18 column (4.6 mm × 250 mm, 5μm) and a 1.0 ml/min flow rate, a 20 μl injection volume, a 40 ^{0}C column temperature, 231 nm UV detection. The mobile phase was comprised of acetonitrile and water in a volume ratio of 35:65.

Solubility studies: Solubility determination of GLA, GLA-PPM, and GLA-PLC were carried out by adding excess of the above samples to 10 ml of distilled water and *n*-octanol in sealed glass vials, respectively. Each experiment was performed in triplicate. After 12 h of shaking in the shaker at 100 rpm under 37 ^{0}C, the solution was centrifuged at 10,000 rpm for 5 min and the obtained supernatants were filtered (0.45 μm, Jinlong, Tianjin, China). The concentration of GLA in the resulting solution was diluted with mobile phase and analyzed by HPLC as described in "2.4.1 Determination of GLA content in phospholipid complex".

Differential scanning calorimetry (DSC): Thermal characteristic of GLA, phospholipid, GLA-PPM, and GLA-PLC were determined using an ATA449-C instrument (NETZSCH, Selb, Germany) under a 10 ml/min stream of nitrogen gas flow. About five milligrams of the samples were heated from 40 ^{0}C to 400 ^{0}C at the heating rate of 10 ^{0}C /min in an open aluminum pan.

Powder X-ray diffraction (PXRD): The PXRD patterns of GLA, phospholipid, GLA-PPM, and GLA-PLC were obtained on a D/MAX 2500-PC X-ray powder diffraction meter (Rigaku Denki, Tokyo, Japan), with a voltage of 50 kV and a current of 40 mA. Data was scanned from 5° to 40° (2θ) with a step size of 0.02°.

Scanning electron microscopy (SEM): The surface morphology of GLA, phospholipid, GLA-PPM, and GLA-PLC were examined using anS-4100 scanning electron microscope (Hitachi, Tokyo, Japan). Samples were sputter coated with gold-palladium and observed at different magnifications.

Preparation of GLA-PLC sustained release pellets

GLA-PLC sustained release pellets were conducted by extrusion-spheronization and fluidized bed coating technology by the following

Table 2: Program and results for orthogonal experimental design.

No.	Fator				Y(%)
	A	B	C	D	
1	1	1	1	1	24.5
2	1	2	2	2	46.4
3	1	3	3	3	88.4
4	2	1	3	3	78.4
5	2	2	2	1	66.6
6	2	3	1	2	86.7
7	3	1	3	2	77.4
8	3	2	1	3	92.3
9	3	3	2	1	55.8
K1	159.3	180.3	203.5	146.9	
K2	231.7	205.3	168.8	210.5	
K3	225.5	230.9	244.2	259.1	
R	24.1	16.9	25.1	37.4	

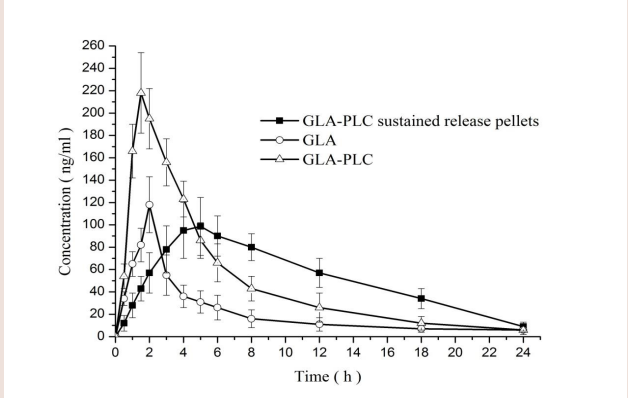

Figure 6: Mean dose-normalized GLA concentration-versus time profiles after administration of free GLA, GLA-PLC, and GLA-PLC sustained-release pellets in beagle dogs. Data are expressed as mean ± SD. (n = 12).

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Table 3: The solubility of GLA, GLA-PPM and GLA-PLC in water and *n*-octanol at 37 ◦C (n=3).

Samples	Apparent solubility (μg/ml)	
	Water	*n*-Octanol
GLA	13.26 ± 2.03	997.34 ± 25.43
GLA-PPM	21.54 ± 3.19	1267.66 ± 42.18
GLA-PLC	182.34 ± 7.13	1589.05 ± 56.65

steps: first, GLA-PLC was mixed with lactose in 1:0.5 weight ratio to get free flowing powder and then sieved by a 60 mesh. Second, the prepared powder was moistened by purified water in order to get soft material. This soft material was extruded by extruder (Guanlian Pharmaceutical Equipment Co., Ltd., Shanghai, China) with dies of 1.0 mm diameter and extrusion speed of 30 rpm. The extrudates were then placed in a spheronizer fitted with a cross-hatched plate rotated for 15 min at a spheronization speed of 1000 rpm. The spheronized pellets were sieved after had been dried in fluidized bed processor (Niro-Aeromatic, Switzerland) at 50 ^{0}C for 25 min.

Finally, the pellets were coated through fluidized bed coating technology as described by PateL SA et al. with some modifications [31]. The coating polymer suspension was prepared by adding16 g triethyl citrate (as plasticizer) and 530 g Kollicoat SR 30 D to 430 g purified water with stirring for 40 min. 55 g talc was added to the above suspension as an anti-tacking agent. The coating suspension was stirred during the coating process. The coating was done at 10% weight gain using a laboratory-scale fluid bed coater (Hanse, Changzhou, China).Experimental parameters were pre-warming of core pellets at 40 ^{0}C for 10 min; atomizing air pressure 1.2 bars; spray nozzle diameter 1.2 mm; air flow rate 90 $m^3 \cdot h^{-1}$; inlet air temperature 50-55 ^{0}C; product temperature 35-40 ^{0}C; spray rate 0.5 $ml \cdot min^{-1}$; post drying at 55 ^{0}C for 15min.

***In vitro* drug release**

The drug release profiles of GLA, GLA-PPM, GLA-PLC, and GLA-PLC sustained-release pellets (all those formulations contained 20 mg GLA were filled into hard gelatin capsules) were studied using Chinese Pharmacopoeia type Ⅲ Apparatus (Tianjin Tianda Tianfa Technology Co., Ltd, Tianjin, China), paddle method with big vessel, at the paddle rotation speed of 50 rpm in 900 ml dissolution medium, at 37 ± 0.5□. At the predetermined times, 1 ml aliquot was withdrawn, filtered (0.45 μm, Jinlong, Tianjin, China), and analyzed by reversed-phase HPLC method as described in "2.4.1 Determination of GLA content in phospholipid complex". 1.0 ml of fresh dissolution media pre-warmed to 37 ^{0}C 0.5 ^{0}C was replaced into the dissolution medium after each sampling. All the experiments were performed in triplicate.

Pharmacokinetic studies in dogs

The *in vivo* pharmacokinetic study described here was reviewed and approved by the Nanjing Medical University Institutional Animal Care and Use Committee (No. 2019-0306) and adhered to the "Principles of Laboratory Animal Care". Tofacilitate administration, GLA, GLA-PLC, and GLA-PLC sustained release pellets were filled into hard gelatin capsules, each dosage contained 20 mg GLA.

An open label, randomized, three-period crossover experiment design was adopted to evaluate the pharmacokinetics of the prepared capsules. Twelve male beagle dogs weighting 10 ± 1 kg were divided into three group and tested under fasted state (with free access to water). The prepared capsules were administered to beagle dogs in the morning with 60 ml of tap water by an oral gavage. After dosing, the dogs were returned to metabolism cages with free access to water. They were provided with standard food after the 12 hours sampling time point.

A total of 5 ml of the whole blood samples were taken from the jugular vein via 20 gauge needle of the dogs before and at the following time points after dosing: 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h. Plasma was obtained by centrifuging for 10 min at 4000 rpm, and was stored in a freezer at -20 ^{0}C before analysis.

The samples were extracted using the following procedure: plasma (1.0 ml) was mixed with 50 μl oridonin methanol solution (0.5 μg/ml) as an internal standard, and 6.0 ml ethyl acetate in a 10 ml plastic centrifuge tube, centrifuged at 4000 rpm for 10 min to precipitate the proteins and the supernatant layer was evaporated in a rotary centrifugal vacuum evaporator. The residue was reconstituted in 100 μl mobile phase and 20 μl of the resulting solution was analyzed by HPLC-UV method, as described in the section "Determination of GLA content in phospholipid complex".

Analysis of data

The pharmacokinetic parameters were calculated by DAS 2.0 software. All values were expressed as the mean ± SD. Statistical analysis was carried out using Student's t-test. Significant difference was regarded as $p<0.05$.

Results and Discussion

Preparation of GLA-PLC

A good amount of aprotic organic solvents can be used as reaction solvents to prepare phospholipid complex, for instance, methanol, ethanol, tetrahydrofuran, acetone and so on [32-33]. In this study, as a way to reduce the harm to human body or our environment, more safety anhydrous ethanol was chosen as the solvent during the process of GLA-PLC.

From the single factor experiment we can know that when the reaction time further increased, there was no obviously difference in the drug-phospholipid combination ratio (The combination ratio were 31.2%, 62.4%, 72.8%, 91.3%, 91.5%, and 91.2% for 0.5 h, 1.0 h, 1.5 h, 2.0 h, 3.0 h, and 4.0 h, respectively.). On the other hand, due to the thermal stability of phospholipid, when the reaction temperature rose over 60 ^{0}C, the combination ratio was declined. Moreover, the combination ratio was declined when the GLA concentration exceeded 10 mg/ml. This phenomenon was due to the excessively high concentration of the two material in the system, which may result in insufficient contact of GLA with phospholipid in the solvent. The combination ratio was increased with the increase of phospholipid proportion. The experimental data were 45.6%, 85.8%, 89.4%, and 91.3% for GLA and phospholipid in 1:0.5, 1:1, 1:2, and 1:3 molar ratios, respectively.

The optimal formulation and processing parameters to obtain the maximum combination ratio of GLA-PLC were selected by orthogonal experiment design. Theoretically, the optimum conditions for the preparation of GLA-PLC were A2B3C3D3.The results are shown in Table 2. In order to validate it, three batches were performed using

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Table 4: Pharmacokinetic parameters of GLA in beagle dogs (n= 12) after oral administration of the GLA, GLA-PLC, and GLA-PLC sustained release pellets.

	GLA	GLA-PLC	GLA-PLC sustained release pellets
C_{max} (ng/ml)	118.2 ± 25.2	218.2 ± 36.3#	98.6 ± 26.5*
T_{max} (h)	2.0 ± 0.5	1.5 ± 0.6#	4.5 ± 1.0*
$MRT_{(0-\infty)}$ (h)	7.8 ± 1.4	7.2 ± 1.5#	11.6 ± 1.5*
$AUC_{(0-\infty)}$ (ng/ml h)	716.7 ± 108.2	1567.4 ± 146.3#	1485.6 ± 104.8*
Relative bioavailability (%)	100	218.7	207.3

#*P < 0.05 compared with GLA.

the optimized preparation conditions (reaction temperature 40 ^{0}C, reaction time 3 h, concentration 15 mg/ml, GLA: phospholipid 1:2). The combination ratio of each batch of GLA-PLC was 94.6%, 95.3%, and 95.0%, respectively.

Characterization of GLA-PLC

Differential scanning calorimetry (DSC): DSC is a fast and reliable thermo-analytical technique to obtain distinct information on the polymorphism and crystallinity of the molecular interaction based on a change in the sharp of peak. Figure 2 presents the DSC curves of GLA (A), phospholipid (B), GLA-PPM (C) and GLA-PLC (D). As shown in Figure 2, GLA showed one sharp endothermic peaks at 223 ^{0}C, suggested that it's crystalline state. The phospholipid displays a broad endotherm at 86^0 corresponding to its phase transition. GLA-PPM exhibited two peaks at 86 ^{0}C and 223 ^{0}C corresponding to phospholipid and GLA, respectively. However, the endothermic peaks at 223 ^{0}C was disappeared in the DSC of GLA-PLC (Figure 2 D), demonstrating the occurrence of molecular interactions between GLA and phospholipid. These results indicated that there was no crystallization of GLA in the GLA-PLC, GLA may be dispersed molecularly or amorphously in the phospholipid molecule.

Powder X-ray diffraction (PXRD): Figure 3 showed the powder X-ray diffraction patterns of GLA (A), phospholipid (B), GLA-PPM (C) and GAL-PLC (D). The spectrum of GLA exhibited numerous sharp diffraction peaks, indicating that the GLA was present as a crystalline pattern. No peaks were detected in the spectrum of phospholipid, which illustrated its amorphous nature. The diffraction pattern of the GLA-PPM revealed the characteristic peaks of GLA, suggested that simple physical mixing had no effect on the interaction between GLA and phospholipid. On the other hand, all the crystalline peaks had disappeared in GLA-PLC, indicated that GLA in a phospholipid matrix was either molecularly dispersed or in an amorphous form [34], which was consistent with the result of the DSC.

Scanning electron microscopy (SEM): The representative surface morphology of GLA (A), phospholipid (B), GLA-PPM (C) and GLA-PLC (D) are presented in (Figure 4). GLA appeared as smooth-surfaced rectangular crystalline structures with different size and the similar crystal structure can be observed in the physical mixture. For GLA-PLC, the morphology was changed completely and rectangular crystalline structures were not observed, indicating that GLA was transformed into an amorphous state.

Solubility studies

From the data in Table 3, we can conclude that the solubility of GLA-PLC in water and *n*-octanol increased significantly ($P < 0.01$) in comparison with GLA. The solubility of the GLA-PLC and GLA-PP Min water was about 13.7 times and 1.6 times higher than GLA. This improvement in solubility could be explained by the amorphous form of GLA and the amphipathic nature and solubilizing effect of phospholipid. In addition, the date illustrated that the solubility of GLA-PLC in *n*-octanol was slightly increased than GLA, which in turn might result in improved absorption across the gastrointestinal tract. In short, the solubility of GLA-PLC increased in water and *n*-octanol means that the drug could be well dissolved and absorbed in gastrointestinal tract.

Preparation and *in vitro* drug release of GLA-PLC sustained-release pellets

Enhancement of the drug solubility in the inner compartment aided the control of the drug release by the outer rate-controlling membrane onto the pellets was the main technical route in this article. In order to design the reservoir-type sustained release pellets, the poorly water soluble GLA was prepared into GLA-PLC which were used as the core material for the layering process. For facilitate the preparation of pellets, lactose was added to GLA-PLC to get free-flowing powder, due to the viscous property of phospholipid. Talc was used as lubricants to prevent aggregation between the pellets and improve powder flow properties. The pellets core were prepared by extrusion-spheronization method, which is one of the conventional methods used since years in pharmaceutical industry [35,36].

The cumulative drug release profiles of GLA, GLA-PPM, GLA-PLC, and GLA-PLC sustained release pellets are shown in (Figure 5). GLA was rapidly released from GLA-PLC at each pH dissolution medium. The GLA-PLC showed96%, 95%, 90%, and 90%of cumulative drug release for 60 min at pH 1.2, pH 4.5, pH 6.8, and pH 7.4 dissolution medium, respectively. In contrast, less than 40% drug was released from pure GLA capsule within 12 h in the four different pH dissolution medium. From these findings we can illustrated that upon introduction of GLA-PLC into gastrointestinal tract environment, the GLA-PLC could achieve fast and complete release of GLA. On the other hand, sustained and total drug release profiles were obtained from GLA-PLC sustained release pellets with over 95% drug release for 12 h at each examined dissolution medium.

Pharmacokinetic studies in dogs

The plasma concentration-time profiles of GLA in beagle dogs following the oral administration of GLA, GLA-PLC, and GLA-PLC sustained release pellets are presented in (Figure 6), and the major pharmacokinetic parameters are summarized in Table 4. From the data we can know that the plasma concentration of GLA from GLA-PLC exhibited a higher C_{max} (218.2 ± 36.3 ng/ml, 1.85-fold) in comparison with free GLA (118.2 ± 25.2 ng/ml). $AUC_{(0-\infty)}$ of GLA in GLA-PLC (1567.4 ± 146.3 ng/ml h) was significantly higher than that of the free GLA (716.7 ± 108.2 ng/ml h), with 2.19-fold increased oral bioavailability.

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Additionally, the maximum plasma concentration obtained from GLA-PLC sustained-release pellets were reach at 4.5 ± 1.0 h and decreased more slowly than that of free GLA and GLA-PLC capsule. The $AUC_{(0-\infty)}$ of GLA-PLC sustained release pellets were 1485.6 ± 104.8 ng/ml.h (2.07-fold), in contrast to 716.7 ± 108.2 ng/ml h of free GLA. In the present case, enhanced oral bioavailability of GLA was achieved by phospholipid complex with the relative bioavailability of GLA-PLC and GLA-PLC sustained-release pellets were 218.7% and 207.3% compared with the free GLA, respectively.

Pharmacokinetics findings demonstrated that GLA-PLC and GLA-PLC sustained-release pellets showed better absorption rate, and/or sustained release behavior *in vivo* than free GLA. This may be attributed to the following reasons:

(1) Compared to crystalline state of GLA, an amorphous state of GLA in GLA-PLC had better solubility and *in vitro* drug release profiles of GLA;

(2) Phospholipids being an important component of cell membrane to maintain the fluidity of cell membrane and owing to the amphiphilic nature, the GLA-PLC are considered to penetrate the cell membrane and enter the cytoplasm of living mammalian cells without disturbing the cellular lipid bilayers;

(3)The combination of GLA to phospholipids can enhance the absorption of GLA through lymphatic pathway and may reduce the exposure of the GLA in the gastrointestinal tract and protect the GLA from degradation by the enzymes [37,38].

(4) The coating layer formed a good barrier membrane to control the drug release from the pellets.

Conclusion

In this paper, GLA-PLC and GLA-PLC sustained-release pellets were successfully prepared for the first time. The physicochemical characteristics of GLA-PLC were indicated by DSC, PXRD, and SEM techniques and the studies showed that GLA in a phospholipid matrix was either molecularly dispersed or in an amorphous form. Our research based on solubility and *in vitro* drug release studies clearly demonstrated a higher solubility and complete drug release profiles for GLA-PLC than free GLA. Besides that, GLA-PLC sustained-release pellets exhibited sustained release characteristics with over 95% total release in 12 h. The pharmacokinetic studies in beagle dogs demonstrated that GLA-PLC and GLA-PLC sustained-release pellets showed 2.19-fold and 2.07-fold improvement than the free GLA by oral dosage in beagle dogs. Further, enhanced oral bioavailability, steady plasma concentration, and prolonger T_{max} were simultaneously obtained from GLA-PLC sustained release pellets, indicating a promising way to achieve the optimal oral bioavailability with sustained release property for poorly water soluble drugs.

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