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Research paper

Enhanced encapsulation and bioavailability of breviscapine in PLGA microparticles by nanocrystal and water-soluble polymer template techniques

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ABSTRACT

Poly (lactide-co-glycolide) (PLGA) microparticles are widely used for controlled drug delivery. Emulsion methods have been commonly used for preparation of PLGA microparticles, but they usually result in low loading capacity, especially for drugs with poor solubility in organic solvents. In the present study, the nanocrystal technology and a water-soluble polymer template method were used to fabricate nanocrystal-loaded microparticles with improved drug loading and encapsulation efficiency for prolonged delivery of breviscapine. Breviscapine nanocrystals were prepared using a precipitation-ultrasonication method and further loaded into PLGA microparticles by casting in a mold from a water-soluble polymer. The obtained disc-like particles were then characterized and compared with the spherical particles prepared by an emulsion-solvent evaporation method. X-ray powder diffraction (XRPD) and confocal laser scanning microscopy (CLSM) analysis confirmed a highly-dispersed state of breviscapine inside the microparticles. The drug form, loading percentage and fabrication techniques significantly affected the loading capacity and efficiency of breviscapine in PLGA microparticles, and their release performance as well. Drug loading was increased from 2.4% up to 15.3% when both nanocrystal and template methods were applied, and encapsulation efficiency increased from 48.5% to 91.9%. But loading efficiency was reduced as the drug loading was increased. All microparticles showed an initial burst release, and then a slow release period of 28 days followed by an erosion-accelerated release phase, which provides a sustained delivery of breviscapine over a month. A relatively stable serum drug level for more than 30 days was observed after intramuscular injection of microparticles in rats. Therefore, PLGA microparticles loaded with nanocrystals of poorly soluble drugs provided a promising approach for long-term therapeutic products characterized with preferable *in vitro* and *in vivo* performance.

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1. Introduction

Breviscapine is a flavonoid mixture extracted from the Chinese herb *Erigeron breviscapus* (Vant.) Hand-Mazz, with its main effective constituent (>85%) of scutellarin (Fig. 1). Breviscapine possesses a variety of pharmacological functions, such as protective effects on cardiac hypertrophy, neuroprotective and renoprotective effects, anti-inflammatory and antiapoptosis activities [1]. It has been used in the treatment of ischemic disease, and disorders in blood supply to heart and brain [2]. Approved breviscapine injections and tablets are available in China. Previous studies have demonstrated that breviscapine exhibited low aqueous solubility, poor chemical stability, short biological half-life and rapid elimination rate from plasma [3]. Different breviscapine formulations were proposed as lipid emulsion [4], solid lipid nanoparticle [5,6], or dendrimer for improved bioavailability [7]. All of them, however, require daily administration, and there is a need for development of a long-term delivery system. A sustained release

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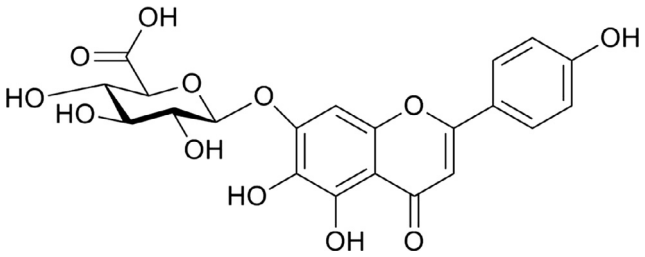

Fig. 1. Chemical structure of scutellarin.

system with long-acting is desired to satisfy the patients' convenience and compliance, as well as to maintain constant therapeutic levels.

Polymeric microparticle formulations have been used to deliver drugs continuously for months. Of the many polymers, poly (lactide-co-glycolide) (PLGA) has been used most widely as a carrier for its biodegradability and biocompatibility [8]. A host of microparticle products based on PLGA has been approved by the U.S. Food and Drug Administration (FDA) [9], such as Lupron Depot® (leuprolide), Trelstar® (triptorelin), Risperidal Consta® (risperidone), and Bydureon® (exenatide). The emulsion methods have been used to make PLGA-based microparticles for encapsulation of a variety of active ingredients [10], including small molecule drugs which are soluble in water or in organic solvents, and peptides or proteins. However, an average of 5% (usually less than 10%) of drug loading is commonly encountered [11,12], and this problem is even worse for those drugs with poor aqueous solubility and low lipophilicity [13]. Many researchers have applied new approaches to address the limitations associated with the conventional emulsion methods. Recently, a novel water-soluble polymer template technique, also known as the hydrogel template method, has been developed for preparation of microparticles with homogeneous size and shape [14] and improved drug loading (up to >30%) as well [15].

Nanocrystals are colloidal dispersions of nano-scale drug particles, which are stabilized by surfactants [16,17]. Nanocrystal technology presents important advantages of high drug loading, a versatile intermediate for different dosage forms and administration routes [18,19]. A host of poorly soluble drugs have been reported on improvement of drug dissolution and oral bioavailability utilizing the nanocrystal technology [20], including breviscapine [21]. However, nanocrystals of poorly soluble drug also have the potential to avoid much loss of drug during fabrication process of PLGA microparticles. Therefore, the nanocrystal technology and water-soluble polymer template method were simultaneously applied in the present study to improve the loading capacity and encapsulation efficiency of breviscapine in microparticles for long-term therapeutic effect. The physico-chemical properties of the obtained microparticles were characterized on their morphology, particles size, DSC and XRPD. Drug distribution in microparticles and *in vitro* release profiles were also analyzed. The data obtained were compared with the microparticles prepared by the emulsion method.

2. Materials and methods

2.1. Materials

PLGA (lactide:glycolide ratio of 50:50; 90 kDa) was purchased from Shandong Institute of Medical Instruments (Jinan, China). Breviscapine (containing 90% of scutellarin) was obtained from Jingzhu Biological Technology Co., Ltd. (Nanjing, China). Poly(vinyl alcohol) (PVA-1788, 44.05 kDa) was supplied by Aladdin Reagent Co., Ltd (Shanghai, China). Poloxamer188 (Lutrol® F68, BASF, Germany) were provided by Chineway Pharmaceutical Tech. Co., Ltd. (Shanghai, China). Rhodamine B was obtained from Tianjin Chemical Reagent Factory (Tianjin, China). All other reagents were either of analytical or of chromatographic grade. Double distilled water was used throughout the experiment.

2.2. Preparation of breviscapine nanocrystals

Nanocrystals were prepared using an ultrasonic-aided antisolvent precipitation method [22]. Briefly, 120 mg breviscapine was dissolved in 300 μl dimethyl sulfoxide (DMSO). The drug solution was quickly injected by syringe into 6.0 ml of 0.5% Poloxamer 188 (W/V) solution under ultrasonication by an Ultrasonic Processor (20–25 kHz, Ningbo Scientz Biotechnology Co. Ltd., China) for 5 min in an ice-water bath. The period of ultrasound burst was set to 90 s with a pause of 1 s between two ultrasound bursts, with the ultrasonic power input of 800 W.

2.3. Characterization of breviscapine nanocrystals

2.3.1. Morphological observation

Samples of fresh nanosuspensions were dropped onto copper grids, dried at room temperature, and then the morphology of nanocrystals was observed by H-7650 transmission electron microscopy at 80 kV (TEM, HITACHI, Japan).

2.3.2. Particle size analysis

A submicron particle analyzer (NICOMP™ 380, PSS Nicomp, Santa Barbara, CA, USA) was used to measure the particle size and size distribution of nanosuspensions. Before analysis, the suspension was diluted with 0.5% Poloxamer 188 solution until the required obscuration was obtained.

2.4. Preparation of breviscapine-PLGA microparticles

The water-soluble polymer templates were prepared from PVA using a previously published method [14,23] and contained circular wells (50 μm diameter and 50 μm depth). The freshly prepared breviscapine nanosuspension was centrifuged at 12,000 rpm for 5 min, and the supernatant was removed. PLGA powder was dissolved in dioxane, and then the polymer solution was vortex-mixed with the nanocrystals. The total amount of PLGA and drug nanocrystals in 2 ml dioxane was fixed at 300 mg. The obtained suspensions were swiped on the water-soluble polymer templates and evenly filled into the wells. The templates were left at room temperature for overnight to evaporate the solvent. The dried templates were put in conical tubes, added water, and gently shaken for 20 min. The released microparticles from completely dissolved templates were collected by filtering (200-mesh sieve, 75 μm) and then centrifuging for 3 min at 4000 rpm. The microparticles obtained were freeze-dried and stored in a refrigerator.

To study the influences of drug forms and fabrication methods on the properties of microparticles, a commonly used oil-in-water (O/W) emulsion method was also used for preparation of breviscapine-PLGA microparticles [24]. Briefly, breviscapine powder was dissolved in DMSO, and then mixed with the PLGA solution in dichloromethane (DCM) at a volumetric ratio of 1:2 to obtain drug-polymer solution. For emulsion method, the breviscapine-PLGA solution was injected into 1% (w/v) PVA solution and homogenized at 8000 rpm for 1 min by an Ultra Turrax T18 homogenizer (IKA, Germany). The initial O/W emulsion was then transferred into 800 ml of 0.5% PVA solution and stirred for 3 h at 40 °C to remove the organic solvents. Finally, microparticles were collected after centrifugation at 4000 rpm for 3 min, washed with water and freeze-dried using a FD-1C freezing dryer (Beijing, China). The

Table 1
Process parameters and particle size of breviscapine microparticles.

Code	Drug form	Organic solvent	PLGA/drug (w/w)	Fabrication method	Particle size (μm)	Span
M1	Solution	DCM:DMSO = 2:1	9.5/0.5	Emulsion	42.70	1.33
M2	Solution	DCM:DMSO = 2:1	9.5/0.5	Template	35.74	0.40
M3	Nanocrystal	Dioxane	9/1	Template	—	—
M4	Nanocrystal	Dioxane	8/2	Template	36.88	0.26
M5	Nanocrystal	Dioxane	7/3	Template	—	—

"—" means not determined.

breviscapine-PLGA solution was also used for microparticle preparation by the water-soluble polymer template method as described above. Table 1 summarized the process parameters used for the breviscapine microparticles.

2.5. Characterization of breviscapine microparticles

2.5.1. Surface morphology

The shape and surface morphology of the microparticles were characterized by a scanning electron microscopy (SEM, JEOL JSM-7500P, Tokyo, Japan). Samples were mounted on an aluminum stub using adhesive carbon tape and sputter-coated with palladium in an argon atmosphere under vaccum.

2.5.2. Particle size analysis

Particle size and size distribution of microparticles were measured by a laser light diffraction (Microtrac X-100, Honeywell, USA) after powder re-dispersion in 10 ml of aqueous solution containing PVA (0.5%, w/v) and Tween 80 (0.1%, w/v). The particle size was expressed as volume weighted mean, and the size distribution was expressed in terms of the Span factor, which is calculated according to the Eq. (1) [25]:

$$\text{Span} = (d_{90} - d_{10})/d_{50} \quad (1)$$

where d_{10}, d_{50}, and d_{90} are particle diameters at percentile 10, 50, and 90 of the distribution curve, respectively.

2.5.3. Drug loading determination

The drug loading (DL) and encapsulation efficiency (EE) were determined by the following method: an accurately weighed sample was dissolved in dioxane, and then diluted with methanol. The concentration of breviscapine was determined by a reversed-phase high pressure liquid chromatography (RP-HPLC) system with a C18 column (250 × 4.6 mm × 5 μm, DIAMONSIL). The mobile phase consisted of 77% triethylamine solution (0.5%, W/V, adjust pH to 4.6 by phosphoric acid) and 23% acetonitrile at a flow rate of 1 ml/min. Breviscapine was quantified by UV detection at 335 nm at a retention time of 4.21 min. The DL and EE were calculated according to the following equations, respectively:

$$\text{DL}(\%) = \frac{\text{Mass of drug in microparticles}}{\text{Mass of total microparticles}} \times 100\% \quad (2)$$

$$\text{EE}(\%) = \frac{\text{DL}}{\text{Theoretical DL}} \times 100\% \quad (3)$$

Results are expressed as mean ± standard deviation (n = 3).

2.5.4. Thermal analysis

Differential scanning calorimetry (DSC) was conducted by a SETSYS-1750 CS Evolution thermogravimetry analyzer (Seraram, France) under nitrogen atmosphere. An accurately weighted amount of sample was introduced in aluminum pan and was submitted to a thermal program from 25 °C to 450 °C at 10 °C/min.

2.5.5. XRPD analysis

X-ray powder diffraction (XRPD) patterns of samples were measured at room temperature. A D/MARX2200/PC diffractometer (Rigaku Co., Tokyo, Japan) using Cu Kα radiation was operated at 40 mA and 40 kV. Intensities were measured in the 3–60° 2θ range at 8°/min.

2.6. Observation of drug distribution inside microparticles

A confocal laser scanning microscopy (CLSM, Olympus FV1000-IX81, Tokyo, Japan) was used to observe drug distribution inside the microparticles. During the preparation of drug nanocrystals and drug-loaded microparticles, rhodamine B was added as an indicator to observe the drug distribution inside microparticles. The samples were excited using a 540 nm laser, and the FV10-ASW 1.7 Viewer software was utilized for image processing.

2.7. In vitro release test

The release rate of breviscapine from microparticles was evaluated in phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBST, pH 7.4). Briefly, 20 mg of dried breviscapine microparticles was suspended in 10 ml PBST and placed in a THZ-100B thermostatic gas bath shaker (Shanghai, China) at 37 °C and continuously stirred at 120 rpm. At various predetermined times, 8 ml of the supernatant was withdrawn after centrifugation at 4000 rpm for 3 min, and replaced with an equal volume of fresh medium. The amount of breviscapine released was measured by the above-mentioned RP-HPLC method.

2.8. Pharmacokinetic study

Twelve Sprague-Dawlay (SD) rats of female, weighing between 200 and 220 g, were provided by the Experimental Animal Center, Ningxia Medical University. The animal experiments were approved by the Ethics Committee, Ningxia Medical University (No. 2013-140). The microparticles of M4 was selected as the test sample and compared with drug suspension. Samples was dispersed into 0.2% sodium carboxyl methyl cellulose solution (containing 0.1% Tween 80, w/v) and sonicated for 5 min to obtain a suspension of pure breviscapine or breviscapine microparticles (with drug content of 5 mg/ml). Rats were randomly divided into two groups and administrated with a single 10 mg/kg dose of breviscapine suspension or microparticles by intramuscular (IM) injection into the left hindlimb. At pre-set time intervals, blood samples of 1 ml were collected via postorbital vein and centrifuged at 3000 rpm for 10 min. One hundred μL of the supernatant was accurately transferred into test tube and mixed with 300 μL methanol by vortexing. The mixture was then sonicated for 15 min followed by centrifugation at 4000 rpm for 10 min. Three hundred μL of supernatant was volatilized under nitrogen gas flow. Finally, 100 μL methanol was added to redissolve the residue and centrifuged at 15, 000 rpm for 10 min, 20 μL supernatant was applied for HPLC analysis of drug content. A mixture of 83.5% triethylamine solution (0.5%, W/V, adjust pH to 4.6 by phosphoric

acid) and 16.5% acetonitrile was used as the mobile phase, and other chromatographic conditions were mentioned above.

The pharmacokinetic results were analyzed by a DAS 3.0 software (Bojia Corp., Shanghai, China). Data were expressed as mean ± S.D. and compared by Student *t*-test. Difference was considered to be statistically significant when $p < 0.05$ or $p < 0.01$.

3. Results and discussion

3.1. Preparation and characterization of breviscapine nanocrystals

The anti-solvent precipitation method was used for preparation of the breviscapine nanosuspensions. The apparent solubility of breviscapine in different solvents was evaluated, and DMSO was selected for the process because it can solubilize larger quantities of the drug. Poloxamer 188 was used as a protective stabilizer to prevent aggregation between the particles.

Fig. 2 presents the size distribution and morphology of breviscapine nanocrystals by dynamic light scattering (DLS) and TEM observation, respectively. The average particle size of breviscapine nanosuspension was 239.4 nm with its polydispersity index (PDI) of 0.356. The crystals were short rod in shape under TEM, with their length ranging from 200 nm to 500 nm approximately, which was in accordance with the results of particle size analysis.

3.2. Preparation and characterization of breviscapine microparticles

When drug solution in DMSO was used in the process, breviscapine-loaded PLGA microparticles were successfully prepared by either O/W emulsion method or template method. However, the microparticles containing breviscapine nanocrystals could be formed only when the template method was applied. The nanocrystals were also tried to be loaded into DCM phase for the emulsion method, but large crystals formed immediately as soon as DCM was mixed with the drug nanocrystals. Breviscapine nanocrystals can be kept in a well-dispersed state only when dioxane was used as the organic phase (see the supporting information, Fig. A.1), but dioxane cannot act as the main organic solvent for the emulsion method as it is miscible with water and may cause shapeless mass instead of microparticles.

The shape and size of the obtained microparticles were characterized by SEM. As shown in Fig. 3a, spherical particles were found for the emulsion method, these microspheres were characterized with small pores on the surface and broad size distribution. The pores were probably formed due to the drug loss and DMSO removal during the solvent solidification process. But for the template method (Fig. 3b–e), all samples showed as dense microdiscs with uniform shape and size (approximately 50 μm in width and 30 μm in height), which was consistent with the previous reports [7,14,26]. Among them, the microparticles prepared by drug-polymer solution (M2, Fig. 3b) were more like tiny cups with wider opening, which may due to the existence of DMSO during the fabrication process. Since DMSO evaporates much slowly than DCM and can partly dissolves PVA, the PVA template becomes softer and then slightly deformed under the pressure of filling operation. The microparticles obtained were deformed accordingly.

The average size of several types of microparticles is presented in Table 1. Samples of M1, M2 and M4 showed a particle size of 42.7 μm, 35.7 μm, and 36.9 μm, respectively. Particle size distribution for microparticles prepared by the template method was obviously narrower than that by the emulsion method, which is in consistent with the SEM results. But for microparticles by the template method, their mean particles size from DLS analysis possessed some differences with those from SEM observation. The deviation might attribute to the analytical error originated from the noncentrosymmetric shape of the microparticles.

A comparison of DL and EE of breviscapine-PLGA microparticles is shown in Fig. 4. When drug-polymer solution was used in the preparation of breviscapine microparticles, the EE was only 53.1% and 48.9% for emulsion and template method, respectively. Approximately half of the loaded drug was lost during the solidification process for emulsion method or the template dissolving process for template method. As expected, the payload was significantly increased by application of drug nanocrystals during the template fabrication approach. Also, a large amount of drug loss was avoided during the fabrication process, the EE are above 80% for all formulations of nanocrystal-loaded microparticles. However, the EE of breviscapine decreased from 91.9% to 82.3%, as the DL increasing from 5.1% to 15.3%, respectively. There would be more drug crystals located in the superficial region of the microparticles, these nanocrystals would be rapidly dissolved into the aqueous solution during the dissolving of PVA template, and then left numerous channels for drug diffusion from inner region of the microparticles. In other words, more nanocrystals means more channels, and it would be easier for drug to escape from microparticles.

As shown in Fig. 4, the application of nanocrystals improved not only drug loading efficiency but also loading capacity of microparticles. For the emulsion method, drug loading was limited by drug solubility in the organic phase. Large aggregates would precipitate out when more drug dissolved into DMSO and mixed with DCM, and microparticles would not take shape with too much DMSO existing in the organic phase. In brief, the improvement of loading capacity and encapsulation efficiency was predominantly attributed to the integrative effect of drug nanocrystals and the template method.

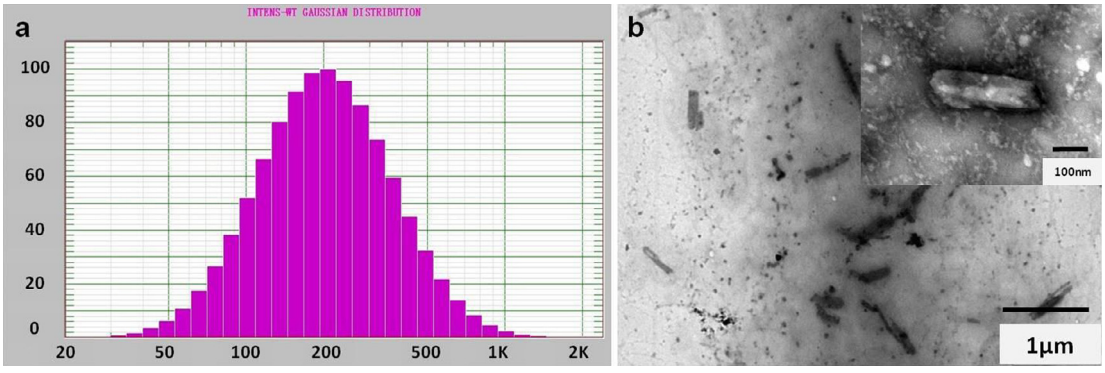

Fig. 2. Particle size distribution (a) and TEM images (b) of breviscapine nanocrystals, scale bar: b, 1 μm (100 nm for the insert).

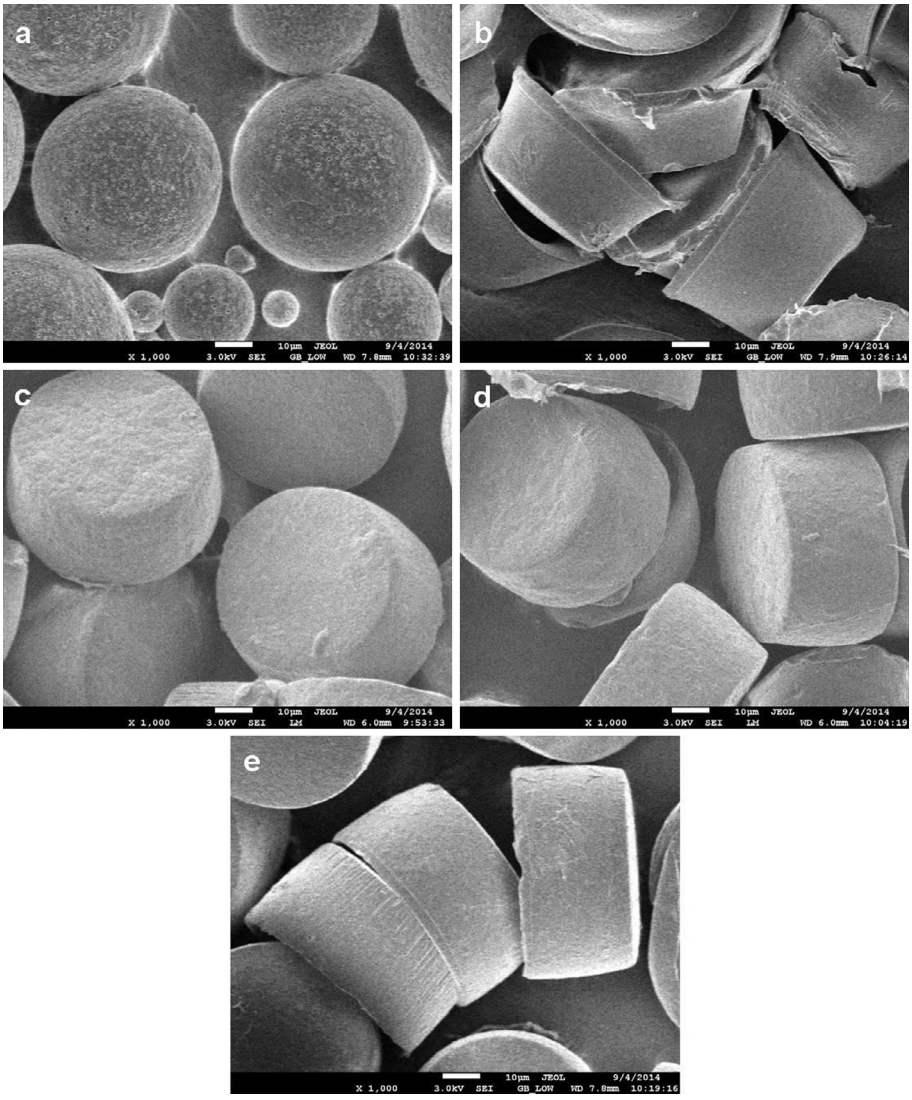

Fig. 3. SEM images of breviscapine-PLGA microparticles by different methods (a: M1; b: M2; c: M3; d: M4; e: M5), scale bar: 10 μm.

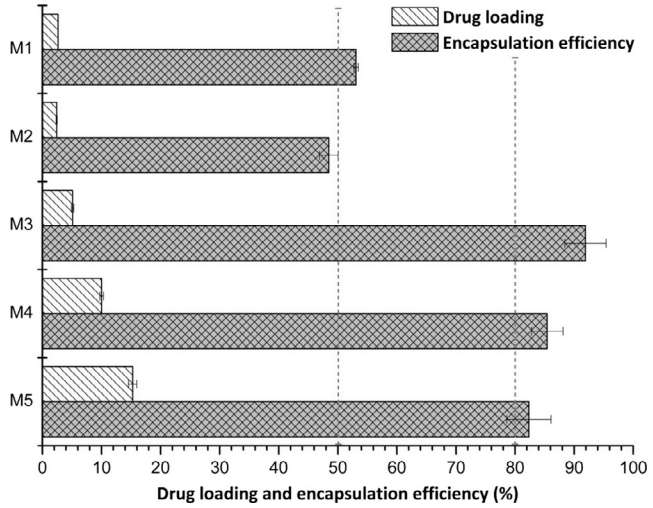

Fig. 4. Drug loading and encapsulation efficiency of breviscapine microparticles by different methods ($n = 3$).

To investigate the effect of fabrication process on thermal behavior of the samples, the DSC thermograms of drug, polymer, physical mixture and microparticles were measured and depicted in Fig. 5. Breviscapine showed an exothermic peak at 196. 8 °C [5], the endothermic peaks at 92.3 °C and 129.4 °C might due to the melting point of some impurities in breviscapine. PLGA experienced a glass transition at 52.9 °C, and decomposed over 250 °C. The *Tg* of the polymer shifted to higher temperature for other samples, which may be assigned to the plasticizing effect of the drug. The exothermic peak of brevascapine was still discernible in the physical mixture, and in the microparticles of M4 and M5. The absence of the drug-related peak in thermograms of other microparticles (M1, M2 and M3) can be either attributed to the low drug loading in these formulations, or the amorphous state of drug inside the microparticles. For all microparticles, the wide decomposition peak of polymer was shifted to over 300 °C, and the decomposition temperature was higher for microparticles loaded with drug nanocrystals than those with drug solution.

X-ray diffractograms of polymer, drug, physical mixture and microparticles are shown in Fig. 6. PLGA was predominantly

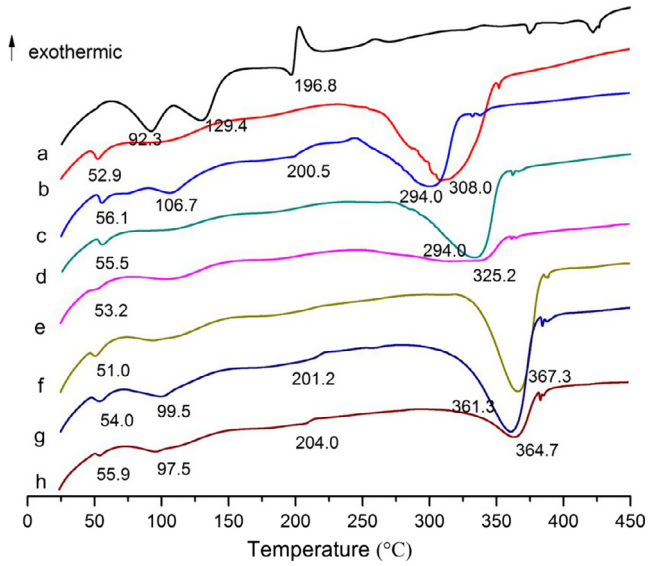

Fig. 5. DSC thermograms of breviscapine microparticles (a: breviscapine, b: PLGA, c: physical mixture, d: M1, e: M2, f: M3, g: M4, h: M5).

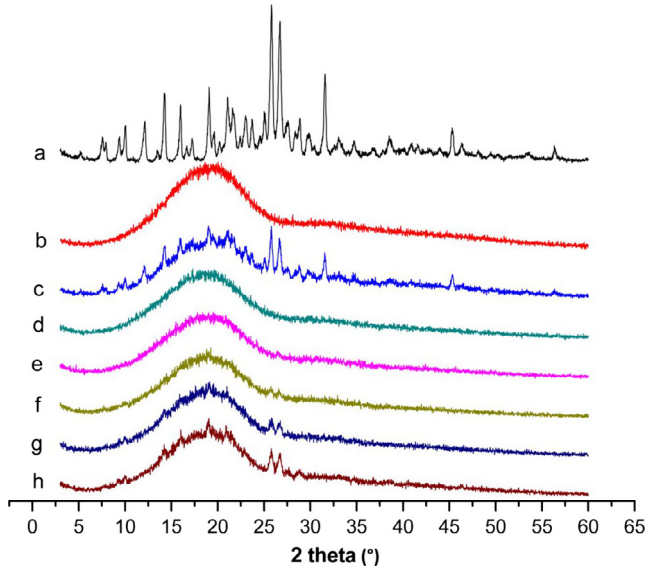

Fig. 6. X-ray diffractograms of breviscapine microparticles (a: breviscapine, b: PLGA, c: physical mixture, d: M1, e: M2, f: M3, g: M4, h: M5).

amorphous as indicated by the slight shift above baseline and lack of any dominant peaks. Numerous sharp peaks at 2θ between 10° and 45° were observed for the bulk breviscapine which suggests the crystalline nature of the drug, and these peaks were maintained in the diffractogram for the physical mixture of drug and polymer (5:95, w/w). However, no crystalline breviscapine was detected due to the absence of intensity peaks in the microparticles prepared by drug solution, indicated an amorphous state or molecularly dispersion of breviscapine [27]. In contrast, several weak peaks characteristic of breviscapine at 26° and 28° were still observable for the microparticles prepared by drug nanocrystals, and their peak intensity was slightly strengthened with the increasing of drug loading. The DSC and XRPD results suggest that the crystallinity of breviscapine was predominantly lost during the process of encapsulation into microparticles, and only partly remained when drug nanocrystals were loaded.

3.3. Drug distribution

Drug distribution inside microparticles was estimated by co-loading a fluorescent probe into the microparticles followed by observation under CLSM. Rhodamine B was added into drug solution in DMSO at a weight ratio of 1:10 (Rhodamine B: drug) and further loaded into the microparticles. CLSM images of the microparticles are shown in Fig. 7. No fluorescence was observed for the blank microparticles (without breviscapine and Rhodamine B, Fig. 7f). The size and shape of drug-loaded microparticles under CLSM was consistent to those under SEM. The images showed that drug was almost uniformly distributed inside microparticles, and the fluorescence was strengthened as drug loading increasing. No significant phase separation of polymer and/or drug was observed.

3.4. In vitro release test

Drug release profiles of breviscapine in five different formulations are shown in Fig. 8. All microparticles exhibited a sustained release pattern consisting of an initial burst, a lag period with slow release and followed by a rapid release phase, which are similar to previous reports [15,28].

The initial burst release was less than 10% except for M5, which loaded with 15% drug nanocrystal and released nearly one half of its total loading after incubation for 4 h. These results suggest that there is a limit for drug loading in breviscapine-PLGA microparticles prepared by nanocrystal-template method, thus any formulations exceeding this limit will possess an initial burst with a significant drug release. A loading of 10% is supposed to be the limit in this study.

After the initial burst, a lag phase with relatively small amount of drug release was observed for all formulations. Differences were found in the duration of lag phase and total percentage of drug release during this phase. About 30% of drug loaded was slowly released for microparticles prepared with drug solution (Fig. 8a), it was less than 20% for those with drug nanocrystals (Fig. 8c). As presented in Fig. 8b and d, the lag phase lasted approximately 28 days for formulations with lower DL (M1, M2 and M3), and 33 days for those with higher DL (M4 and M5).

After the lag phase, drug release was accelerated by PLGA erosion. During the rapid release phase, the release rate of breviscapine from microparticles by solution-template method was higher than that by solution-emulsion method (Fig. 8a), and it also decreased with the increasing of DL in microparticles by nanocrystal-template method (Fig. 8c).

The obtained microparticles were different in shape and/or structure by emulsion or template method, as well as by application of drug solution or nanocrystals. These differences may cause variation in erosion behavior, and therefore varied release profiles among different microparticles. It has been suggested that the primary degradation mechanism of drug-loaded PLGA microparticles were bulk erosion for emulsion method and surface erosion for template method [26]. Therefore, we may speculate from these results that the template technique provides more dense texture for microparticles than the emulsion method. Besides, drug dissolution was required before drug molecules could diffuse out from the nanocrystal-loaded microparticles into the bulk medium.

3.5. Pharmacokinetic study

The drug concentration-time profiles and pharmacokinetic parameters of breviscapine suspension and microparticles following IM administration in rats are shown in Fig.9 and Table 2, respectively.

As illustrated in Fig. 9, a rapid increase in drug serum concentration was detected after IM injection of breviscapine suspension

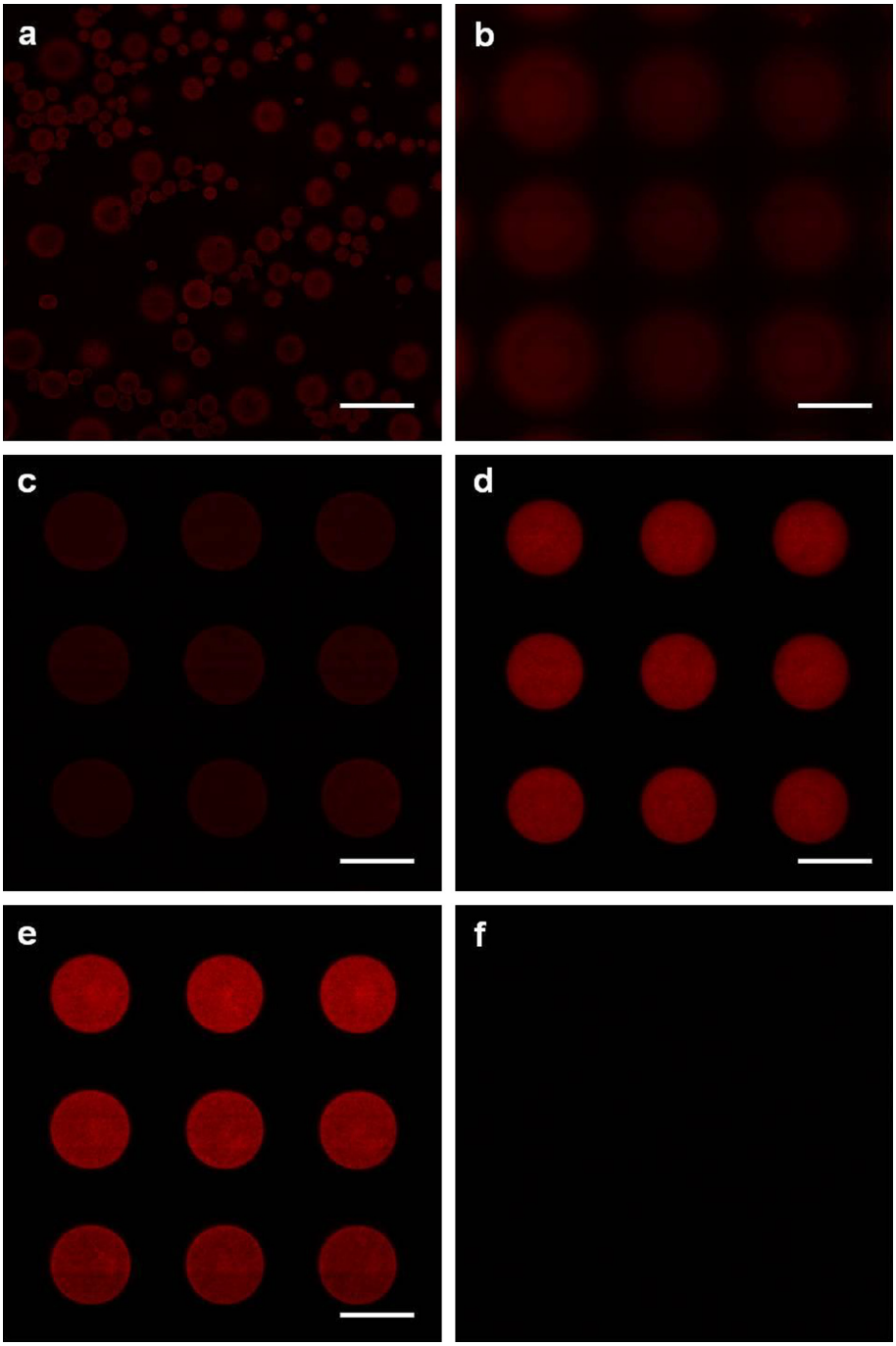

Fig. 7. CLSM images of breviscapine microparticles prepared by different methods (a: M1; b: M2; c: M3; d: M4; e: M5; f: blank microparticles), scale bar: 50 μm.

in rats, with a peak (1.2 μg/ml) occurring at 15 min after the administration followed by a progressive decline to low drug level (0.1 μg/ml) within 24 h. However, the microparticle formulation (M4) showed an initial drug level of 0.70 μg/ml after injected for 4 h. And the drug level fluctuated in the range of 0.4–0.8 μg/ml in the first 35 days, then dropped quickly to 0.02 μg/ml after 38 days. Therefore, the administration of the microparticles loaded with breviscapine nanocrystals could provide a relatively steady drug concentration in plasma at a preferable level for a long period. The microparticle formulation may be injected once every month, instead of daily administration for the conventional formulation.

Compared to the drug suspension, the microparticle formulation lowered C_{max} of breviscapine in rats, and significantly prolonged T_{max}, $t_{1/2}$ and $MRT_{0\text{-}t}$ as well. Moreover, the AUC of breviscapine was dramatically enhanced by over 30-fold for the microparticle group. These results suggested the potential application of breviscapine-PLGA microparticles at low dose and frequency, which further revealing enhanced therapeutic efficacy and better clinical adaptability.

4. Conclusions

In this study, the water-soluble polymer template method was successfully applied to fabricate breviscapine nanocrystal-loaded microparticles with sustained release for a prolonged period. Comparative studies suggested that the obtained microparticles

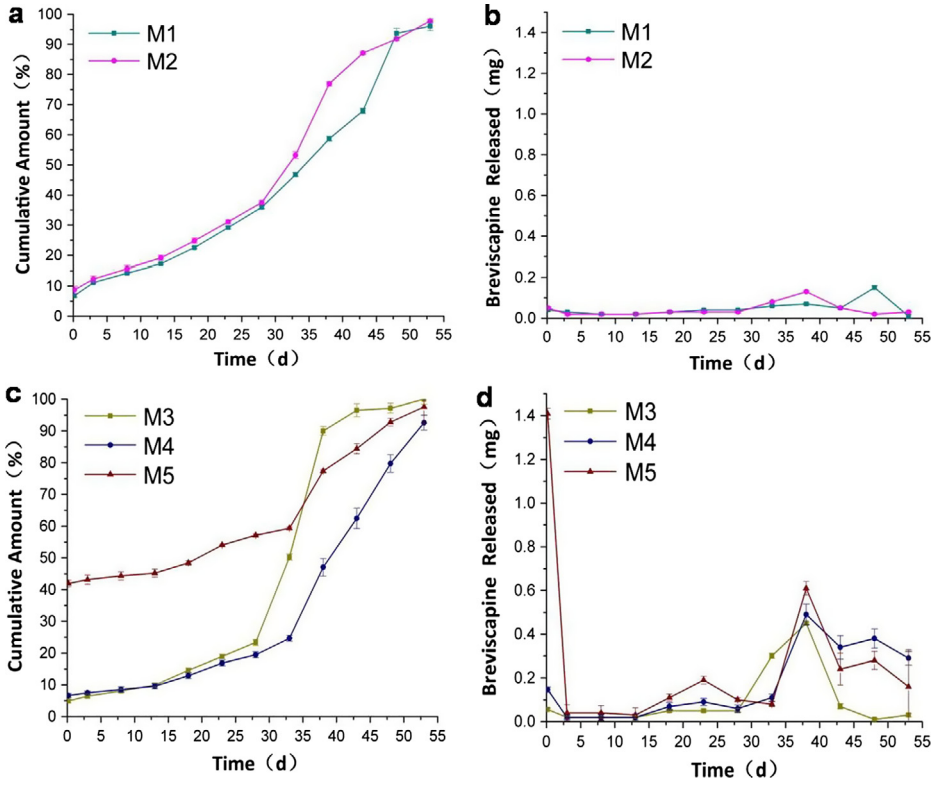

Fig. 8. *In vitro* release profiles in percentage (a, c) and amount (b, d) of breviscapine from PLGA microparticles fabricated by drug solution (a, b) or by drug nanocrystals (c, d) (n = 3).

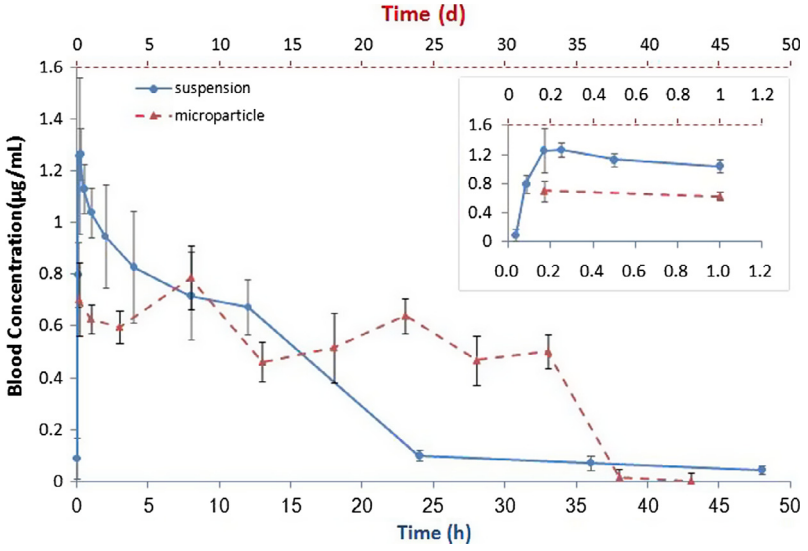

Fig. 9. Plasma concentration profiles of breviscapine in rats after a single intramuscular administration of breviscapine suspension or microparticle formulation (M4) (n = 6).

possessed not only uniform size and shape but also high drug loading and encapsulation efficiency. Breviscapine nanocrystals were dispersed homogeneously and showed substantial influences on the cumulative release profiles of the microparticles. The nanocrystal technology and water-soluble polymer template method simultaneously contributed to the remarkable improvement of drug loading in breviscapine-PLGA microparticles. Pharmacokinetic test in rats indicated that the optimized breviscapine microparticle formulation provided a stable serum drug level for up to one month after a single dose, suggesting its potential application as an efficient long-acting product.

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Table 2
Pharmacokinetic parameters of breviscapine suspension and microparticle formulation following intramuscular administration ($n = 6$).

Parameter	Suspension	Microparticle (M4)
C_{max} (μg/mL)	1.37 ± 0.26	0.82 ± 0.10
T_{max} (h, d)	0.24 ± 0.03	5.762 ± 3.82*
$t_{1/2}$ (h, d)	7.59 ± 1.40	2.75 ± 1.15*
$AUC_{0\text{-}t}$ (μg h/mL, μg d/mL)	16.09 ± 2.47	20.30 ± 1.09*
$MRT_{0\text{-}t}$ (h, d)	10.86 ± 0.49	16.78 ± 0.43*

The unit is h or μg h/mL for the suspension formulation, and d or μg d/mL for the microparticle formulation. C_{max}, maximum concentration; T_{max}, time to peak concentration; $t_{1/2}$, half-value period; AUC, area under the concentration-time curve; MRT, mean retention time.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejpb.2017.02.021.

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