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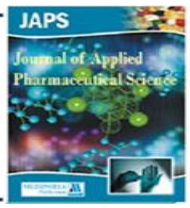

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Improving Oral Bioavailability of Agaricoglycerides by Solid Lipid-Based Self-Emulsifying Drug Delivery System

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ABSTRACT

Agaricoglyceride A (AGA), showed strong activities against neurolysin. The objectives of this study was to prepare solid self-emulsifying drug delivery system (sSEDDS) by spray drying the SEDDS (liquid system) using Aerosil 200 as the inert solid carrier, and to evaluate the enhanced bioavailability (BA) of AGA from sSEDDS. The AGA formulated in the sSEDDS was quickly and completely dissolved within 20min, both in 0.1N HCl and phosphate buffer pH 6.8 dissolution media, whereas AGA powder was significantly less dissoluble. Meanwhile, the sSEDDS formulation was stable for at least 90days at room temperature. the plasma levels of AGA in the solid SEDDS group at the dose level (15mg/kg) remained detectable for up to 1.5 h after the oral dose. After oral administration to rats, a significant increase ($P<0.0001$) in the C_{pmax} and $AUC_{0\rightarrow 24\ h}$ were observed in the sSEDDS group when compared with the AGA powder group. Furthermore, AGA-loaded sSEDDS exerted significant antinociceptive properties and alleviated pain insults in mice. The results suggest that the sSEDDS could be considered and further evaluated for the oral delivery of lipophilic poorly soluble drugs for which an oral route of administration is desirable.

Keywords: Solid self-emulsifying; oral bioavailability; lipophilic drugs; antinociceptive properties; Agaricoglyceride A

INTRODUCTION

The agaricoglycerides are a new class of fungal secondary metabolites that constitute esters of chlorinated 4-hydroxy benzoic acid and glycerol (Fig.1). They are produced in mycelial cultures of several mushrooms of which some are edible. The main active principle, agaricoglyceride A (AGA), showed strong activities against neurolysin, a protease involved in the regulation of dynorphin and neurotensin metabolism, and even exhibited moderate analgesic *in vivo* activities in an *in vivo* model (Stadler et al., 2005). However, the clinical advancement of this promising compound is hampered by its poor water soluble and highly lipophilic that result in a low oral bioavailability. Therefore, suitable formulation approaches need to be developed to improve solubility and bioavailability of this poorly soluble compound.

In recent years, much attention has been paid to solid self-emulsifying drug delivery systems (sSEDDS), which have shown lots of reasonable successes in improving oral bioavailability of poorly soluble drugs (Gursoy et al., 2004; Kang et al., 2004; Chen et al., 2008). This novel drug delivery system combines the advantages of liquid SEDDS with those of a solid dosage form and overcomes the limitations associated with liquid formulations (Tang et al., 2008; Wang et al., 2009). sSEDDS produces oil-in-water emulsions of droplet size less than 200 nm upon mild agitation in aqueous media (such as gastrointestinal fluids (Cui et al., 2008; S.V.R et al., 2008). These fine droplets of emulsions have the advantage of presenting the drug in dissolved form with a large interfacial surface area for drug absorption, which results in enhanced more uniform and reproducible bioavailability(S.V.R et al., 2008). In the present study, we prepared sSEDDS containing phosphatidylcholine (PC) for the delivery of AGA from the established composition of SEDDS by spray drying technique and evaluated its bioavailability (BA) in rats.

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MATERIALS AND METHODS

Materials

Agaricoglycerides was produced in the Pharmaceutic Laboratory of Shandong University of Traditional Chinese Medicine, China (Chunchao, 2010). Briefly, mycelia of *Coprinus comatus* were separated from the culture fluid by filtration and extracted twice with acetone in an ultrasonic bath. The extract was filtered, and the acetone was removed *in vacuo* to yield an aqueous residue. This residue was diluted with tap water and subsequently extracted three times with EtOAc. The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo* to yield an oily residue.

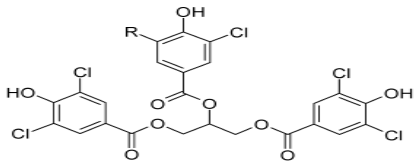

Fig. 1: Chemical structures of Agaricoglyceride.

METHODS

Preparation of AGA-Loaded sSEDDS

Based on the pilot studies, the blank SEDDS were prepared by mixing of 30% Miglyol® 812 (oil), 60% Cremophor® RH40 and Tween80 (surfactant, 2:1) and 10% Transcutol®P (cosurfactant) at 50 ^{0}C with a magnetic stirrer. Then AGA and Aerosil 200 (1000mg) suspended in 150ml ethanol were dissolved in the blank SEDDS with stirring until forming an isotropic mixture. The mixture was then kept at room temperature and equilibrating for 24h.Then it spray dried with a ZPG mini spray dryer B-190 apparatus (Changzhou, China) under the following conditions: inlet temperature, 60°C; outlet temperature, 35°C; aspiration, 85%; feeding rate of the suspension, 5ml/min. The final drug content of the solid SEDDS was 5.33% w/w ratio.

Reconstitution Properties of the sSEDDS

The emulsification time of the SEDDS or S-SEDDS formulations was evaluated according to the way described in detail by Stadler et al (Stadler et al., 2005).In brief, either SEDDS (250 µl) or S-SEDDS (500 mg) was intro introduced into 500 ml of distilled water at 37 °C under gentle agitation by a standard stainless steel dissolution paddle rotating at 50 rpm. The emulsfIcation time was assessed visually as reported previously. All experiments were carried out in triplicates. Droplet size was determined by Zetasizer Nano ZS (Malvern Instruments, UK) with dynamic light scattering particle size analyzer at a wavelength of 635 nm and at a scattering angle of 90° at 25 °C. All studies were repeated three times, and the values of z-average diameters were used. The z-average diameter, also referred to as the harmonic intensity-weighted average hydrodynamic diameter, of the emulsions was derived from cumulated analysis by the Automeasure soft-ware (Malvern Instruments, UK). Zeta potential of the emulsion formed after addition of SNEDDS or sSEDDS into 0.1 N HCl solution was measured using Zetasizer Nano ZS (Malvern Instruments, UK).

Dissolution studies of the sSEDDS

Dissolution profiles of the sSEDDS were determined using the paddle method at 100 rpm with 900ml of 0.1N HCl and phosphate buffer pH 6.8 as dissolution media performed at 37 °C. Samples (3 ml) withdrawn, at fixed time intervals of 10 minutes, were filtered using a 0.45 µm membrane filter and were assayed for AGA by HPLC. Crude AGA powder was used as control standard.

Stability Studies

To evaluate the chemical and physical stability of sSEDDS, it was placed in the glass vial protected from light with or without rubber-stopper at 25 °C with 75% relative humidity for 3 months. Samples were removed at fixed time intervals of 30 d and determined for droplet size, AGA content.

Evaluation of antinociceptive properties of sSEDDS

Kunming strain of Swiss mice weighing 20-22g, were purchased from the Experimental Animal Center, Shandong University. The female and male mice were maintained at room temperature under alternating natural light/dark photoperiod, and had access to standard laboratory food and fresh water *ad libitum*. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals. Care was taken to minimize discomfort, distress, and pain to the animals. The mice were randomly divided into three groups (n=10, each group). AGA powder (20mg/kg) and sSEDDS (376mg/kg, re-dispersed in 1ml of distilled water) were orally administered to two groups respectively, while another group was given intranasal vehicle (phosphate buffered saline, PBS). Formalin test, which causes a local tissue injury to the paw, has been used as a model for tonic pain and localized inflammatory pain. For this, 20 µL of a 1%-formalin solution was injected into the right hind paw of mice, and the licking time was recorded after the first 5 min (1st phase, corresponding to a direct chemical stimulation of nociceptors) and after 20 min (2nd phase, involving inflammation), for 5 min each time. Animals were pretreated with drugs 60 min before intraplantar formalin injection (Fasmer et al., 1985).

Pharmacokinetic studies

Wistar rats (2 months old and weighing 225 ± 25 g) were used in the study. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals. Care was taken to minimize discomfort, distress, and pain to the animals. The pharmacokinetic (PK) profile of AGA from SNEDDS, S-SNEDDS, or AGA was investigated in rats with a dose equivalent to 15mg/kg of AGA and other formulations were orally administered to the rats after dispersion in 20 ml of water. Following oral administration, 2.5 ml of blood was collected from either right marginal ear vein at predetermined time intervals, and 1 ml of plasma was separated by centrifuging blood samples at 3000 g for 15 min. Plasma samples were stored at -20 °C until further analysis. The area under the drug concentration–time curve from zero to infinity ($AUC_{0\rightarrow 24\ h}$) was calculated using the trapezoidal rule. The maximal plasma concentration of drug (C_{pmax})

and the time to reach maximum plasma concentration (T_{max}) were directly obtained from plasma data. The data from different formulations were compared for statistical significance by ANOVA.

Statistical analysis

Statistical data analysis was performed using the student t-test with $p < 0.05$ as the minimal level of significance unless indicated otherwise. All values were expressed as the means ± S.D.

RESULTS AND DISCUSSION

Reconstitution Properties of the sSEDDS

sSEDDS formulation disperse quickly and completely when subjected to aqueous environment under mild agitation (see below). The efficiency of self-emulsification can be estimated by measuring the rate of emulsification and the droplet size distribution (J.H. et al., 2010). As shown in table 1, emulsification time was 21 ± 2 s for SNEDDS, while it was 41 ± 5 s for the sSEDDS formulations. It is much related to their hydrophilic–lipophilic balance (HLB) value of surfactant and co-surfactant.

It has been reported that any change in interfacial film influences the surface curvature of the droplet leading to differences in the droplet size (Balakrishnan et al., 2009; Gershanik et al., 2000; L.L. et al., 2005). As shown in table 1, the z-average droplet sizes for liquid SEDDS and sSEDDS systems were 122.8 and 152.6 nm, respectively. Both the mean droplet size and size distribution (polydispersity index) of the emulsion remained almost unchanged in spite of the conversion from a liquid to a solid state, indicating that the spray-drying process did not have a remarkable influence on the emulsfication performance.

Table 1: Reconstitution properties of the sSEDDS

Groups	SNEDDS	sSNEDDS
Emulsification time (seconds)	21 ± 2	41 ± 5
Zeta potential	+1.53 ± 0.30	+1.04 ± 0.31
Droplet size (nm)	122.8 nm	152.6 nm
Polydispersity index (PDI)	0.162 ± 0.012	0.211 ± 0.023

Values are shown as means ± SD.

Dissolution studies of the sSEDDS

Cumulative percent of AGA dissolved into the aqueous medium are important criteria that govern the quality of the solid-state self-emulsified dosage form. The extent of dissolution is dependent on the reversible attraction and surface adsorption of AGA and the oily formulation onto the adsorbing powder. As shown in Fig. 2, for the crude powder of AGA, only less than 1% of the drug was dissolved in the phosphate buffer pH 6.8 medium, and 9% of the drug was dissolved in the 0.1N HCl medium during the period of determination. This dissolution characteristic of crude AGA powder in different media may be a result of its intrinsically weak acidity. On the contrary, the drug dissolution profiles of GA-loaded solid SEDDS in both phosphate buffer pH 6.8 and 0.1N HCl showed complete dissolution within 20 min. The mechanism of sSEDDS exert a high solubilization capacity could be explained that it provides ultra low interfacial tensions and large o/w interfacial areas, leading to the incorporation of poorly water-soluble pharmaceuticals inside the fine oil droplets (Xi et al., 2009).

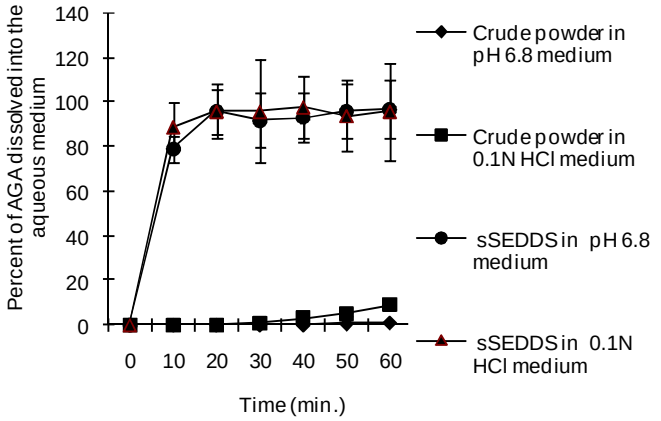

Fig. 2: Dissolution Profiles of AGA Powder and CAGA-Loaded SEDDS in Phosphate Buffer pH 6.8 and 0.1N HCl. Values are shown as means ± SEM. (n=6)

Stability of the sSEDDS

The AGA-loaded SEDDS was stable at room temperature for 90days as shown in Fig. 3. Mean particle size was constant both. Moreover, the content of AGA was maintained in the range of 90-115% and showed no significant difference. It was also seen that the spraying dried formulation was stable to humidity. Thus, these studies confirmed the stability of AGA-loaded SEDDS.

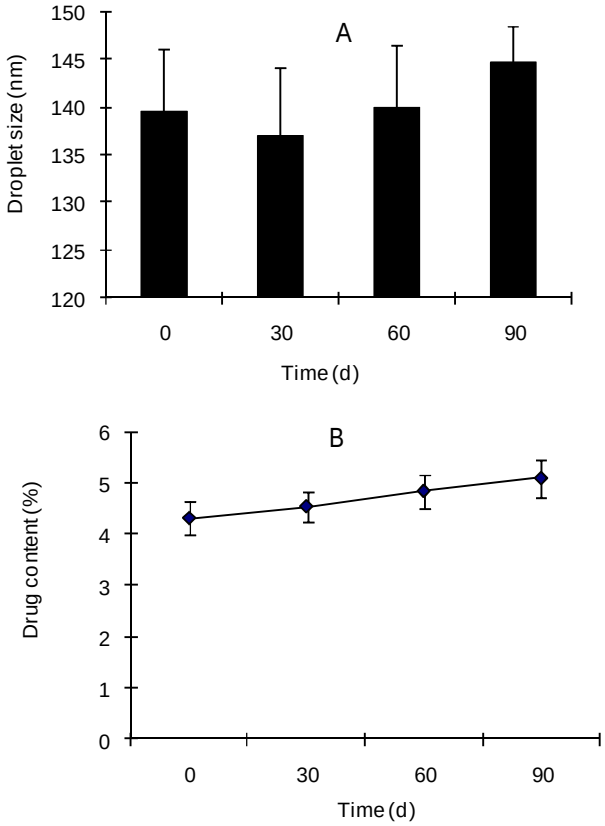

Fig. 3: Particle size (A) and content of AGA (B) stability of the sSEDDS.

Evaluation of antinociceptive properties of sSEDDS

AGA showed strong activities against neurolysin, and even exhibited moderate analgesic *in vivo* activities in an *in vivo* model (1, 9). In order to improve oral bioavailability, we prepared sSEDDS for the delivery of AGA from the established composition of SEDDS. The formalin test is constituted by two distinct phases. Central acting analgesics, such as morphine, inhibit both phases. Peripheral acting drugs, such as non-steroid anti-inflammatory and corticosteroids, inhibit only the second phase (Hunskaar et al., 1987). The present results revealed that both phases of the response were significantly inhibited following oral delivery sSEDDS. On the contrary, neither phases of the response were significantly inhibited following oral delivery AGA (Table 2).

Table 2: Antinociceptive effect of AGA-loaded sSNEDDS in mice submitted to the formalin test. (n = 10)

Groups	Licking time (s)		Inhibition (%)	
	1st phase	2nd phase	1st phase	2nd phase
Control	61.22± 3.3	30.3 ± 3.2	-	-
AGA-loaded sSNEDDS	45.09± 4.9	20..4± 3.3	26.3%	32.7%
AGA powder	59.22± 3.4	28.3± 3.1	3.2%	6.6%

Values are shown as means ± SD. *p < 0.05 vs. control group, **p <0.01 vs. control group.

The antinociceptive animal testing indicates that AGA-loaded sSEDDS were able to improve antinociceptive effect of AGA.

Pharmacokinetic studies of sSEDDS

The main reason for the enhanced drug oral bioavailability by sSEDDS is the excellent efficiency of SEDDS in improving the drug solubility and increasing the dissolution rate (Ghosh et al., 2006; Liu et al., 2009). SEDDS provides ultra low interfacial tensions and large o/w interfacial areas, resulting in a more efficient drug transport through the intestinal aqueous boundary layer, leading to an improvement in oral bioavailability (Patel et al., 2009). In the present study, an in vivo absorption study was undertaken to determine whether or not the enhanced solubility and in vitro dissolution of AGA in a sSEDDS could increase the GI absorption of drug after oral administration. Table 3 shows the pharmacokinetic variables measured in this study. The total plasma concentrations of drug after oral administration of AGA powder could not be detected (at the dose level of 15mg/kg) due to its very small absorption in rats. On the contrary, the plasma levels of AGA in the solid SEDDS group at the dose level (15mg/kg) remained detectable for up to 1.5 h after the oral dose. A significant increase ($P<0.0001$) in the C_{pmax} and $AUC_{0\rightarrow 24\ h}$ were observed in the sSEDDS group when compared with the AGA group. Thus, the higher plasma concentrations of AGA in sSEDDS were contributed by improving the solubility of drug. These results suggested that oral AGA absorption in the sSEDDS was significantly increased compared to that of the power formulation. The current data are consistent with the findings of many other poor solubility drugs (Lu et al., 2008; Ghosh et al., 2006).

CONCLUSION

In this study, sSEDDS was prepared for the delivery of AGA by spray drying using Aerosil 200 as the inert solid carrier. This sSEDDS preserved the self-emulsification performance of the liquid SEDDS and gave a faster in vitro dissolution rate than the crude powder both in 0.1N HCl and phosphate buffer pH 6.8 dissolution media. Furthermore, it allowed a significant improvement in AGA bioavailability in rats after oral administration compared to a powder suspension formulation. AGA-loaded sSEDDS exerted significant antinociceptive properties and alleviated pain insults in mice. The results suggest that the sSEDDS could be considered and further evaluated for the oral delivery of lipophilic poorly soluble drugs for which an oral route of administration is desirable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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