CAW™ Enteric-Coated Cycloastragenol Capsules: Enhanced Drug Stability and Oral Bioavailability

ABSTRACT

The objectives of the present work were to use enteric-coated capsule shell for cycloastragenol capsules. The influence of the enteric-coated capsule on gastric stability in vitro drug release and drug stability was evaluated. Furthermore, the bioavailability of the enteric-coated cycloastragenol capsules in beagle dogs was also performed. The enteric-coated capsules exhibited significant improvement of gastric stability and drug stability compared to the common capsules. Moreover, the AUC values of enteric-coated cycloastragenol capsules were greater than that of the common ones. It was concluded that the using enteric-coated capsule shell for cycloastragenol improved the drug stability and oral bioavailability.

1. INTRODUCTION

Cycloastragenol (CAG) is a secondary metabolite isolated from Radix Astragali. Present in all known Astragalus spp., CAG (Fig. 1) is both a triterpene aglycone and the most common genuine aglycone in the bioactive triterpenoid saponins called astragalosides (ASTs) (Rios and Waterman, 1997). Although CAG and the ASTs are found in all tissues of the Astragalus shrubs, the highest concentrations are localized in the roots (Yu et al., 2007b). Ten out of the eleven ASTs found in the root of A. membranaceus contain CAG as the aglycone, including AST IV, the primary characteristic and main bioactive AST present in the roots of this species (Kitagawa et al., 1983; McKenna et al., 2002; Sevimli-Gur et al., 2011; Verotta and El-Sebakhy, 2001; Yu et al., 2007a; Zhou et al., 2012). CAG, AST IV, and other related molecules isolated from Astragalus spp. have also been identified as small-molecule telomerase activators, substances that can induce the elongation of telomeres, the protective DNA sequences at the terminal ends of chromosomes (de Jesus et al., 2011; Fauce et al., 2008; Harley et al., 2011; Yang et al., 2012; Yung et al., 2012; Zhou et al., 2012).

Cycloastragenol has the instable three-membered ring in 9, 10 position. Compared to other triterpene aglycone, it is more instable to heat, light, and acidic medium. Especially, the drug degrades rapidly in acid medium. Thus, an enteric coating must be applied to the solid dosage form to prevent the drug from degradation in stomach and allow drug release in small intestine.

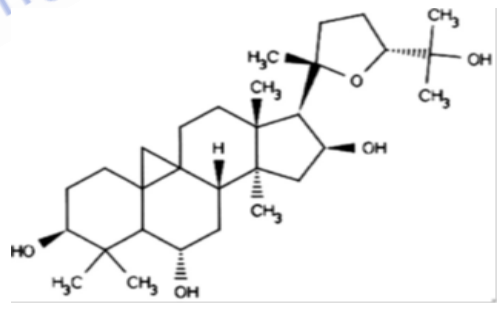

Fig. 1. Structure of cycloastragenol.

The use of enteric-coated capsule may influence the drug stability and bioavailability greatly which offer many advantages as follows (Siepmann F et al., 2008; Kranz H et al., 2009): (1) facilitated adjustment of desired drug release patterns, mechanical properties, and drug release mechanisms, (2) improved storage stability, and (3) the possibility to develop novel strategies for site-specific drug delivery within the gastrointestinal tract. To date, few reports have indicated that enteric-coated capsule are utilized for cycloastragenol formulation.

Thus, the aim of the present work was to (a) use enteric-coated capsule shell for cycloastragenol capsules; (b) evaluate the influence of the enteric-coated capsule on gastric stability, in vitro drug release and drug stability; and (c) study the bioavailability of the enteric-coated cycloastragenol capsules in beagle dogs.

2. METHODS

2.1 In Vitro Drug Release

After immersed in acidic medium (0.1MHCl) for 1 h, the drug release from the capsules was measured in a paddle USP apparatus (75 rpm, 37°C, 900 mL (pH 6.8)). At specific time intervals, samples were withdrawn and analyzed using a HPLC assay described below.

2.2 Gastric Stability

The gastric-stability study was performed by exposing the capsules in acid medium (500 mL, 0.1 M HCl) for 1 h. The amount of drug degradation was determined by a HPLC method described below.

2.3 Bioavailability in Dogs

The bioavailability of the enteric-coated capsules and the common ones were assessed and compared in dogs in a randomized cross-over study. The wash out period was 1 week. Six male beagle dogs (8–10 kg) used in the experiments received care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals.

The dogs were fasted 12 h before administration. The two kinds of capsules filled with cycloastragenol pellets was orally administered to the dogs at a dosage of 1 mg/kg. All of the formulations were administered with water of 20 mL. Blood samples (2 mL) were collected from saphenous vein into heparinized tubes at the following time points: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 12 h. The heparinized blood samples were immediately centrifuged at 4,000×g for 10 min in a desktop centrifuge (Anke TGL-16G, China), and the plasma was separated and transferred to microcentrifuge tubes. The plasma samples were frozen at ?18°C until analysis. Frozen plasma samples were prepared by a procedure reported by Ito et al., which the drug samples was measured by a validated HPLC method described below.

2.4 HPLC Analysis

The cycloastragenol concentrations in plasma were determined using an HPLC assay reported by Dugger and coworkers (25). Briefly, a 1-mL aliquot of plasma was extracted with 6 mL of extraction solution containing internal standard (megestrol acetate, 100 ÿg/mL in methyl-t-butyl ether). After mixing and centrifugation, the organic phase was removed and evaporated to dryness under nitrogen stream. The residue was reconstituted in 100 mL of methanol and centrifuged at 10,000×g for 5 min, and then 50 ÿL of the supernatant liquid was injected onto the HPLC system.

The HPLC system consisted of a Waters 2414 Refractive Index Detector and an Empower workstation. The separations were performed at 30°C using a 250 mm×4.6 mm column (DiamonsilTM C18). The mobile phase was consisted of 70% methanol and 30% water and was pumped at a flow rate of 1.0 mL/min. The eluent was detected by RID detector and corresponding peak areas were recorded.

2.5 Data Analysis

Pharmacokinetic parameters were calculated by noncompartmental analysis based on statistical moment theory using Microsoft Excel 2003. The pharmacokinetic parameters, such as maximum plasma concentration (Cmax) and time of maximum concentration (Tmax), were obtained directly from the plasma concentration-time plots. The area under the plasma concentration-time curve up to the last time (t) (AUC0–t) was calculated using

The results were expressed as mean±standard deviation. One-way analysis of variance was performed to assess the statistical significance of differences among samples. Results with $P<0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effect of Enteric-coated capsule shell on Drug Release

The Enteric-coated capsule shell produced significant effect on the drug release (Fig. 2). At the first 30 min, the drug release was gradually decreased. The gastric stability of cycloastragenol was very poor, indicating that all of the drug was degraded when the cycloastragenol were immersed in acid medium for 10 min. Thus, the testing for the gastric stability and drug release of excellent without damage in the first 20 min. Thus, it was explained that the Enteric-coated capsule shell led to an increase in length of diffusion pathways and increasing the time required for the drug to diffuse through the coating membrane.

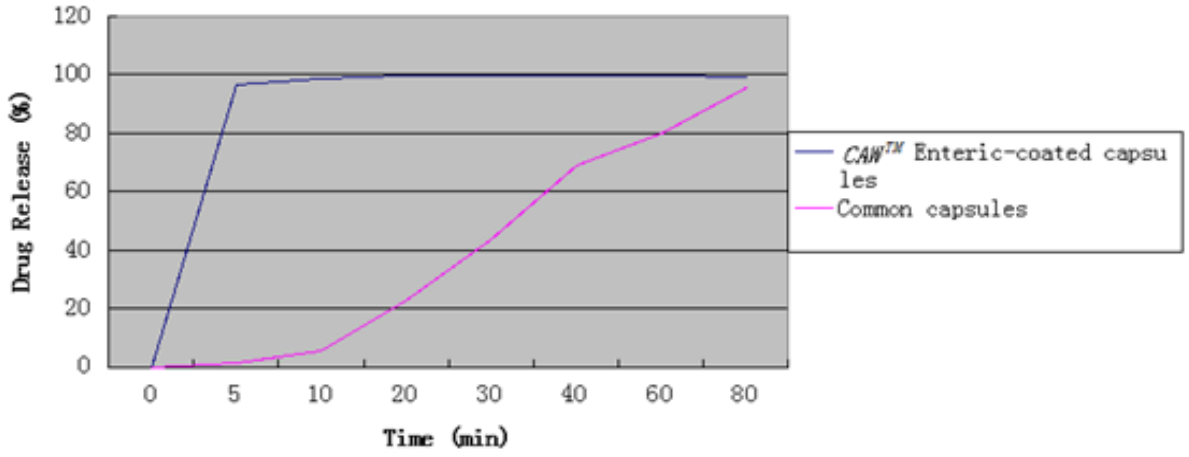

Fig. 2. Effect of *CAW™* Enteric-coated capsule shell on the drug release from the coated pellets.

3.2 Gastric Stability

As shown in Fig. 3, the Enteric-coated capsule significantly improved the gastric stability of cycloastragenol. During the acid phase, the swelling of the capsule shell, water penetration into the core, drug dissolution, and subsequent diffusion were contributed to the drug release. The Enteric-coated capsules were beneficial to reducing the drug release in acid medium and improving the gastric stability of cycloastragenol.

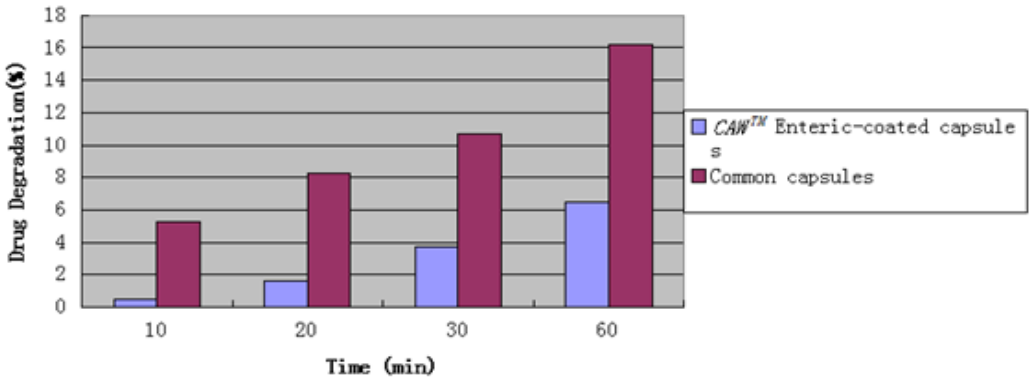

Fig. 3. Effect of *CAW™* Enteric-coated capsule on the drug degradation

3.3 Pharmacokinetics in Dogs

Mean plasma cycloastragenol concentration versus time profiles following a single oral dose of the two formulations are shown in Fig. 4. Mean values of the pharmacokinetic parameters are summarized in Table I.

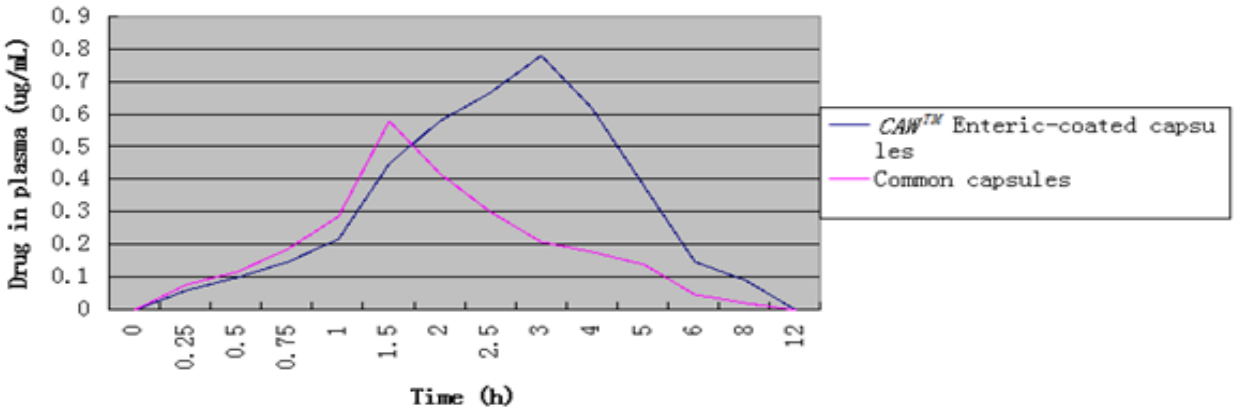

Fig. 4. Plasma cycloastragenol concentrations after oral administration of cycloastragenol cupsules at a dose of 1 mg/kg in dogs. (n=6)

The Tmax/Cmax of cycloastragenol from Enteric-coated capsules was 3.00±0.59 h/0.78±0.12 ÿg mL?1. In the case of common capsules, the Tmax/Cmax was 1.50±0.35 h/0.58±0.10 ÿg mL?1, which Tmax differed from the values obtained from the Enteric-coated capsules (P< 0.05). Interestingly, the AUC values of Enteric-coated capsules were 1.89±0.14 ÿg h mL?1, which was greater than that of the common capsules(1.23 ± 0.17 ÿg h mL? 1) (P<0.05). The relative bioavailability calculated by the Enteric-coated capsules AUC to the common capsules AUC was 153.56±34.45. The enhanced absorption was ascribed to the fact that the Enteric-coated capsules could improve the gastric Stability of cycloastragenol. Additionally, compared to the common capsules, the Enteric-coated capsules had different curves of drug release, which led to variation in disintegration site in the small intestine and a time lag and then affected the bioavailability of the drugs. Thirdly, beagle dogs were widely used to study the bioavailability of oral formulations, since the dimensions of the GI tract are similar enough to permit the administration of dosage forms. However, the gastric pH in fasted dogs was not similar to that in humans and the fluctuation of pH must be considered. Due to low basal gastric acid secretion, the gastric pH in fasting dogs fluctuated (2.7–8.3), and it was as high as the pH of its duodenal content, which would produce significant effect on the drug absorption of preparations with pH-dependent release. If the enteric-coated capsules were administered to the fasted dogs, the enteric films might be damaged and led to premature drug release, drug instability, and decreasing bioavailability. The drug release of conventional enteric-coated dosage forms always occurred in the distal small intestine, resulting in a delayed response to medication and decreased the drug bioavailability.

Table I. Pharmacokinetic Parameters of Cycloastragenol After Oral Administration

PK parameters	Common capsules	Enteric-coated capsules
C_{max} (μg/mL)	0.58±0.10	0.78±0.12
T_{max}	1.50±0.35	3.00±0.59*
AUC_{0-t} (μg h/mL)	1.23 ± 0.17	1.89±0.14*

* Statistically higher than common capsules (P<0.05)

4. CONCLUSIONS

The enteric-coated capsules for cycloastragenol improved the gastric stability and oral bioavailability significantly. Moreover, the AUC values of enteric-coated cycloastragenol capsules were greater than that of the common ones. It was concluded that the using enteric-coated capsule shell for cycloastragenol improved the drug stability and oral bioavailability.

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