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RESEARCH PAPER

Formulation and Pharmacokinetic Studies of Acyclovir Controlled-Release Capsules

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ABSTRACT

Acyclovir controlled-release capsules (CRCs) were prepared by a three-step process: (1) melt granulation of acyclovir; (2) coating of granules with ethylcellulose; (3) incorporation of coated granules into hard gelatin capsules. In vitro release experiments showed that the main factors affecting the release rate were the mean particle size of the acyclovir raw material and the amount of coating material applied. Release of acyclovir from the capsules was in accordance with the Higuchi equation. Pharmacokinetic studies in dogs after oral administration of acyclovir controlled-release capsules showed that the formulation was successful in providing slow release of acyclovir and was superior to a commercially available controlled-release formulation.

Key Words: *Acyclovir; Controlled-release capsules; Dog; Pharmacokinetics*

INTRODUCTION

Acyclovir, previously known as acycloguanosine, has potent inhibitory effects on viruses of the herpes group, particularly herpes simplex virus (HSV) (I and II) and herpes zoster varicellaous virus. It also combines inhibitory effects on hepatitis B virus with very low toxicity to mammalian host cells (1). Various reports indicate that acyclovir is as effective as or superior to other antiviral agents with lower

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host toxicity and milder side effects (2). Unfortunately, acyclovir has a short half-life (2–3 h), and the oral dosage form must be taken 5 times daily, which is very inconvenient for patients. The aim of this study was to develop a controlled-release formulation of acyclovir that could be taken twice daily.

Flamel Technologies previously developed a controlled-release capsule (CRC) of acyclovir using a patented micropump technique. The formulation contains ethylcellulose-coated pellets prepared by a two-step process involving wet granulation and coating. In the wet granulation step, water/isopropanol (50/50 w/w) was used as the wetting agent, and polyvinylpyrrolidone (PVP) was used as a binder and osmotic agent (3).

Recently, melt or thermoplastic granulation has been widely used to obtain powder agglomeration by the addition of a binder such as polyethylene glycol that melts or softens at a relatively low temperature (4–9). When melted, the action of the binder is similar to that occurring in a wet granulation process. As a "one-step" operation, melt granulation offers several advantages compared to conventional wet granulation in that it is inexpensive and safe since the liquid addition and the subsequent drying phase included in the wet granulation process are not necessary. Granules prepared by melt granulation demonstrate better physical strength and have a smoother surface than those obtained by wet granulation, and they are suitable for further operations, such as coating.

In the present investigation, the melt granulation process was employed to prepare granules using polyethylene glycol 6000 (PEG6000) as the hydrophilic binder. During the melt granulation in a high-shear granulator, the thermal energy required for granule formation was generated by friction. It is well known that the solubility of acyclovir in water is increased when PEG6000 is used as a melt binder. Ethycellulose was used as a coating material in this study to control the release rate of acyclovir.

EXPERIMENTAL

Materials

Acyclovir was purchased from Shanghai No. 12 Pharmaceutical Factory, Shanghai, China. PVP (K30 grade) and PEG6000 were purchased from Shanghai Chemical Reagent Company, Shanghai, China. Ethylcellulose (Ethocel, 45 cP) was received as a gift from Colorcon Company, Shanghai, China. Castor oil was purchased from Jinzhou Oil Chemical Factory, Jinzhou, China.

Preparation of Controlled-Release Capsules

Melt Granulation

Acyclovir (100 g) with a particle size of 3–5 μm was mixed with PEG6000 (20 g) in a high-shear mixer (JLL-30A Superblender, Heygey Electrical Company, Zhongshan City, China) at 1200 rpm for 20 min. The temperature in the blender increased to more than 60°C during mixing, which was sufficient to promote the formation of granules. After cooling to room temperature, granules with a particle size of 400–600 μm were then coated with ethylcellulose.

Coating of Granules and Preparation of Capsules

To coat acyclovir granules, ethylcellulose (20 g), PVP (1.5 g), and castor oil (2.0 g) were dissolved in acetone (200 g). Isopropranol (25 g) and magnesium stearate (3.0 g) were then added to produce a suspension of magnesium stearate that was agitated throughout the coating process.

Acyclovir granules (100 g) as prepared above were put into a coating pan at 120 rpm and heated for 10 min. The coating suspension was then sprayed onto the granules at a rate of 1 ml/min from an injection nozzle (diameter 0.3 mm) fed by a peristaltic pump operating at a pressure of 20 psi. The coated pellets of acyclovir were then dried for 30 min in the coating pan, followed by 8 h of drying at 40°C. The volume of coating solution applied was varied to prepare pellets with different amounts of coating.

The acyclovir content in the coated pellets was assayed by ultraviolet (UV) spectroscopy at 252 nm. A weighed quantity of coated pellets containing about 40 mg acyclovir was added to hot distilled water (20 ml) and shaken ultrasonically for 15 min to dissolve the acyclovir. The suspension was then diluted to 250 ml and filtered. An aliquot (5 ml) was diluted to 100 ml, and the absorbance was determined using a UV spectrophotometer (model 752C, Shanghai Analytical Instrument Factory, Shanghai, China).

Controlled-release capsules were prepared by filling gelatin capsules (size 1) with sufficient coated pellets to provide a dose of 200 mg of acyclovir per capsule.

In Vitro Release Study

In vitro release of acyclovir from controlled-release capsules was investigated using the basket apparatus (ZRS-4 dissolution tester, Tianjin University Radio Factory, China) at 100 rpm. The release medium was 900 ml of distilled water maintained at 37°C. Aliquots of release medium (5 ml) were removed at 0.5, 1, 2, 4, 6, 8, 10, and 12 h after the start of the experiment. The amount of acyclovir released was determined by measurement of the absorbance at 252 nm.

Pharmacokinetic Study in Dogs

Dogs ($n = 12$) were purchased from Nanjing Medical University Experimental Animal Center. The dogs were divided into two groups and administered either 2×100 mg commercial acyclovir tablets (CT, Kangna Pharmaceutical Co., Ltd., Hubei, China) or 1×200 mg acyclovir controlled-release capsule (CRC; containing 16% coating material) by the oral route. Blood samples (3 ml) were collected initially and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, and 36 h after dosing. Serum was separated by centrifugation at 1500*g* for 15 min and analyzed for acyclovir content.

High-Performance Liquid Chromatographic Assay

The concentration of acyclovir in dog serum was determined by high-performance liquid chromatography (HPLC). The chromatographic system consisted of a Waters 515 HPLC pump, a Waters 2487 dual-wavelength absorbance detector, and Jiangshen Chromatography Station Software (Jiangshen Chromatography Co., Ltd., Dalian, China). Chromatographic separations were achieved on a Lichrosphere RP18 column (4.6 mm × 250 mm, 5 μm (Huaiyin Hanbang Sci-Tech Co., Ltd., China) maintained at 40°C and a Waters guard column (4.0 mm × 4.0 mm). The mobile phase was a mixture (4 : 96 v/v) of methanol and aqueous 0.05 M sodium acetate solution containing 0.0025 M sodium 1-heptanesulfonate. The flow rate of the mobile phase was maintained at 1.0 ml/min, and the effluent was monitored at 254 nm. Serum samples for analysis (0.5 ml) were mixed with 0.5 ml of 7% $HClO_4$ (vortex mixer for 5 min) and centrifuged at 5000*g* for 15 min. Then, 20 μl of the resulting supernatant was injected into the HPLC.

The chromatograms of acyclovir (Fig. 1) show a stable baseline and good resolution between acyclovir and endogenous material in plasma. The assay was linear in the range 0.25–0.0 μg/ml. The mean regression equation for five replicate calibration curves on different days was $C = 0.009194\,As - 201.035171$ ($r = 0.99995$). The mean relative recovery of acyclovir in dog plasma is shown in Table 1. Precision and accuracy, investigated by replicate analysis of control standards in dog plasma, were satisfactory, as shown in Table 1.

Data Analysis

The release profiles of acyclovir from controlled-release capsules were fitted with zero-order, first-order, or Higuchi equations. Values of

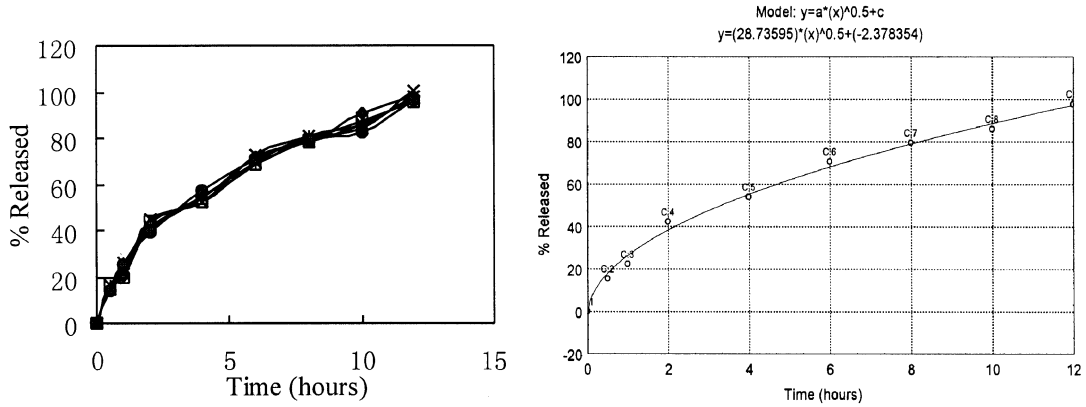

Figure 1. In vitro release profiles of acyclovir controlled-release capsules. Left: release curves ($n = 6$). Right: simulated curve of mean data.

Table 1

Precision and Accuracy of High-Performance Liquid Chromatographic Analysis of Acyclovir in Dog Plasma

Target Concentration (ng/ml)	Within-Day Precision			Between-Day Precision			Recovery
	$\overline{As} \pm$ SD	CV %	Accuracy %	$\overline{As} \pm$ SD	CV %	Accuracy %	Mean $\pm$ SD ($n = 5$)%
250	47523 ± 2150	4.52	−5.64	47502 ± 2411	5.07	−5.72	99.9 ± 1.9
1250	164764 ± 6395	3.88	5.10	155524 ± 4601	2.96	−1.70	103.1 ± 1.2
10,000	1079254 ± 37752	3.50	−2.78	1108183 ± 52257	4.72	−0.12	91.0 ± 1.6

Accuracy percentage was defined as [(Measure concentration − Target concentration)/ Target concentration] × 100%.
CV, coefficient of variation.

pharmacokinetic parameters of acyclovir in dogs, including C_{max}, t_{max}, AUC_0, $AUMC_0$, MRT, Cl/F, and $t_{1/2}$ were calculated using standard noncompartmental methods. All data were evaluated by analyses of variance (ANOVAs).

$$AUC_{0-\tau} = \sum (C_i + C_{i-1})(t_i - t_{i-1})/2$$

$$AUMC_{0-\tau} = \sum (C_i t_i + C_{i-1} t_{i-1})(t_i - t_{i-1})/2$$

$$MRT = \frac{AUMC_{0-\tau}}{AUC_{0-\tau}}$$

$$Cl/F = D/AUC_{0-\tau}$$

$$t_{1/2} = 0.693/\lambda_z$$

RESULTS AND DISCUSSION

Release Profiles

Release profiles of acyclovir controlled-release capsules containing pellets with 16% dry coating material are shown in Fig. 2. The data fitted to zero-order, first-order, and Higuchi equations gave the following regression equations: $Q = 7.50 \times t + 15.73$ $(r = 0.9640)$; $Q = 26.72 \times \exp(-0.12 \times t)$ $(r = 0.9064)$; $Q = 28.14 \times t^{1/2} - 2.53$ $(r = 0.9969)$, respectively. It can be seen that the release is best described by the Higuchi equation.

Effect of Particle Size on Release Rate

Acyclovir with mean particle sizes of 17.2 ± 5.5 and 176.3 ± 104.6 μm was used to prepare acyclovir controlled-release capsules and investigate the effect of mean particle size on the release rate of acyclovir. The in vitro release fitted by Higuchi equations is shown in Fig. 3. Regression equations for the release from capsules containing small and large particles were $Q = 21.37 \times t^{1/2} - 6.23$ $(r = 0.9978)$ and $Q = 28.14 \times t^{1/2} - 2.53$ $(r = 0.9913)$, respectively. This

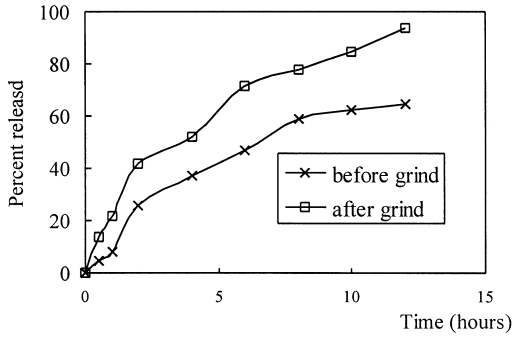

Figure 2. Effect of mean particle size of acyclovir on the release rate.

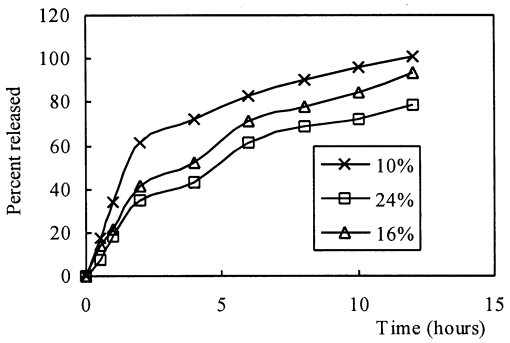

Figure 3. The effect of applied amount of coating material on the release rate.

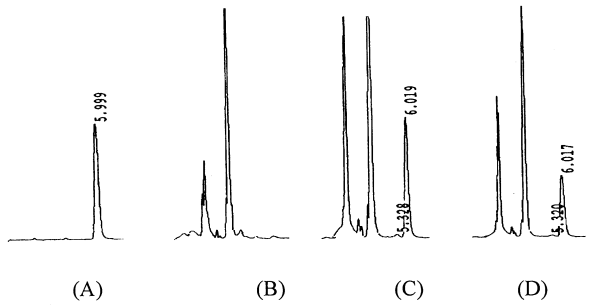

Figure 4. High-performance liquid chromatograms of (A) standard solution of acyclovir, (B) blank dog plasma, (C) blank dog plasma with acyclovir added, and (D) dog plasma sample after taking acyclovir controlled-release capsules.

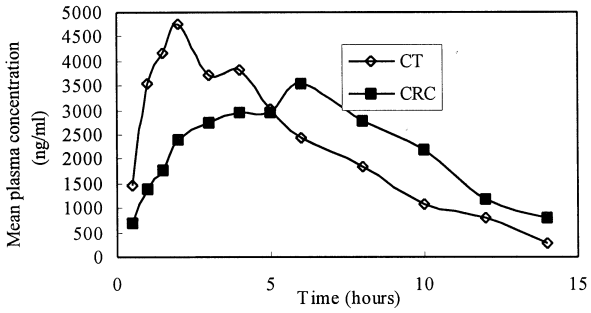

Figure 5. The mean plasma concentration-time profiles for the single-dose study.

Table 2

Pharmacokinetic Parameters Obtained After a Single Oral Dose (200 mg) of Commercial Tablets (CT) and Controlled-Release Capsules (CRC) of Acyclovir

	CT		CRC		
	Mean	SD	Mean	SD	*P*
C_{max} (ng/ml)	5608	2350	4345	1983	.341
T_{max} (h)	2.33	1.03	4.67	1.63	.014
$t_{1/2}$ (h)	3.96	1.01	5.14	3.85	.484
$AUC_{0\sim\tau}$ (ng.h/ml)	29998.13	7916.33	30721.65	13758.99	.913
MRT (h)	5.03	0.68	6.77	1.05	.007
Cl/F (l/h)	7.06	1.80	7.52	2.86	.742

means that a decrease in particle size results in faster dissolution.

Effect of Coating Amount on Release Rate

Acyclovir granules with 10%, 16%, and 24% coating material were encapsulated in size 1 gelatin capsules. The in vitro release of acyclovir from capsules with 10%, 16%, and 24% coating material were fitted to the Higuchi equation and gave the following regression equations: $Q = 29.99 \times t^{1/2} - 4.93$; $Q = 28.14 \times t^{1/2} - 2.53$; $Q = 24.52 \times t^{1/2} - 3.80$, respectively. The data are shown in Fig. 4. It is clear that a low content of coating material results in faster release, with over 60% being released in 2 h. The high content of coating material (24%) resulted in incomplete release, with less than 80% being released in 12 h. A level of 16% of coating material resulted in optimum controlled release (i.e., 40% in 2 h, 50% in 6 h, 75% in 8 h, and 95% in 12 h).

Pharmacokinetics of Acyclovir Controlled-Release Capsules in Dogs

Mean plasma concentration-time profiles in dogs administered either commercial acyclovir tablets (CT) or the acyclovir controlled-release capsules (CRC) prepared in this study are shown in Fig. 5. Table 2 summarizes the pharmacokinetic parameters obtained. The results show that (1) C_{max}, $AUC_{0-\tau''}$ Cl/F for CRC and CT were not significantly different; (2) the t_{max} for CRC was significantly greater than that for CT; and (3) the MRT of CRC was significantly greater than that for CT.

The fact that these two parameters are both larger for CRC shows it is a superior controlled-release formulation than that of the commercially available tablet. The relative bioavailability of CRC was 102% that of CT.

CONCLUSION

Acyclovir controlled-release capsules were prepared by encapsulating acyclovir pellets prepared by melt granulation and coating them with ethylcellulose. The melt granulation or one-step granulation process provides a convenient method of preparing coated granules. In vitro release experiments show that mean particle size of acyclovir raw material and amount of coating material applied are the main factors that affect the release rate. The release data were well described by the Higuchi equation.

Pharmacokinetic studies in dogs after oral administration of acyclovir controlled-release capsules or commercially available tablets showed the release profile of CRC capsules was superior to that of CT tablets. The efficacy and safety of this dosage form are currently being evaluated in clinical pharmacokinetic trials.

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