Influence of a Niosomal Formulation on the Oral Bioavailability of Acyclovir in Rabbits

Received: May 21, 2007; Final Revision Received: July 14, 2007; Accepted: July 18, 2007; Published: December 14, 2007

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ABSTRACT

The purpose of this research was to prepare acyclovir niosomes in a trial to improve its poor and variable oral bioavailability. The nonionic surfactant vesicles were prepared by the conventional thin film hydration method. The lipid mixture consisted of cholesterol, span 60, and dicetyl phosphate in the molar ratio of 65:60:5, respectively. The percentage entrapment was ~11% of acyclovir used in the hydration process. The vesicles have an average size of 0.95 μm, a most probable size of 0.8 μm, and a size range of 0.4 to 2.2 μm. Most of the niosomes have unilamellar spherical shape. In vitro drug release profile was found to follow Higuchi’s equation for free and niosomal drug. The niosomal formulation exhibited significantly retarded release compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability of acyclovir in rabbits after a single oral dose of 40 mg kg−1. The average relative bioavailability of the drug from the niosomal dispersion in relation to the free solution was 2.55 indicating more than 2-fold increase in drug bioavailability. The niosomal dispersion showed significant increase in the mean residence time (MRT) of acyclovir reflecting sustained release characteristics. In conclusion, the niosomal formulation could be a promising delivery system for acyclovir with improved oral bioavailability and prolonged drug release profiles.

KEYWORDS: Acyclovir niosomes, oral acyclovir, bioavailability of acyclovir.

INTRODUCTION

Acyclovir is a synthetic acyclic purine nucleoside analog that is currently used for the prevention and treatment of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections.1 The oral bioavailability of acyclovir is low, variable, and species dependent.2 The pharmacokinetic properties of acyclovir are well established.3 The effects of dosage size on the extent of oral absorption are not well understood. Some reports suggest that absorption from the gastrointestinal tract may be a saturable, dose-dependent process.4 In contrast, another study reported a relative constancy in the urinary recovery of unchanged drug and in the bioavailability calculated from urinary excretion data, concluding that the net absorption of acyclovir is nearly proportional to the dose.2 Acyclovir is categorized as a class III drug according to the Biopharmaceutical Classification System (BSC) because of its high solubility and low permeability.5 The US Food and Drug Administration (FDA) guidelines6 reported that a drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL or less of the aqueous media over the pH range of 1 to 7.5. The intrinsic solubility of acyclovir was 1.2 mg/mL as measured by the acid-base titration method.7 Acyclovir has dissociation constants (pka) of 2.34 and 9.23 and partition coefficient (P-octanol) of ~0.023.8 This low partition coefficient may reflect the low membrane permeability of acyclovir. According to the FDA guidelines,6 the drug is considered highly permeable when the extent of absorption in human is determined to be >90% of the administered dose in comparison to an intravenous reference dose. In humans, acyclovir showed poor and variable oral bioavailability (15%-30%), probably due to the relatively low lipophilicity of the drug.2 Thus, the rate-limiting factor in acyclovir absorption is its membrane permeability. The inclusion of absorption-enhancing excipients in the formulations can enhance the drug bioavailability. Trials that have been made to improve the oral bioavailability of acyclovir concerned mainly with chemical modification of the drug.9,10 Luengo et al11 studied the pharmacokinetics of different preparations of acyclovir with β-cyclodextrin and found that β-cyclodextrin showed no significant effect on the oral drug bioavailability. This finding is because the effect of cyclodextrin was mainly on the solubility of the lipophilic drug not the permeability of the hydrophilic drug. Encapsulation of acyclovir in lipophilic vesicular structure may be expected to enhance the oral absorption and prolong the existence of the drug in the systemic circulation. Niosomes are nonionic surfactant vesicles that are well recognized as drug delivery vehicles. Niosomes can carry hydrophilic drugs by encapsulation, are quite stable, and require no special conditions for production or storage. Preliminary studies indicate that niosomes may increase the absorption of certain drugs from the gastrointestinal tract following oral ingestion.12
In the present study, acyclovir-loaded niosomes were formulated and evaluated for their in vitro as well as in vivo characteristics in an attempt to improve the oral bioavailability of the drug. The in vivo evaluation of acyclovir niosomes in comparison with free drug solution was conducted in rabbits after a single oral dose.

**MATERIALS AND METHODS**

**Materials**

Acyclovir was obtained from Glaxo Welcom (Cairo, Egypt). Acyclovir sodium (freeze-dried acyclovir as the sodium salt) was purchased from Welcom (London, UK). Dicetyl phosphate (DCP), sorbitan monostearate (span 60), and sephadex G-25 were purchased from Sigma Chemical Co (St Louis, MO). Cholesterol (CHOL) was from BDH (Pool, UK). Triton X-100 was obtained from PARK (Northampton, UK). All other chemicals used were of analytical grade.

**Preparation of Acyclovir Niosomes**

The nonionic surfactant vesicles were prepared by the conventional thin film hydration method. Cholesterol, span 60, and dicetyl phosphate (47.5 mg CHOL, 47.5 mg span 60, and 5 mg DCP) in a molar ratio of 65:60:5 were dissolved in 2.5-mL chloroform-methanol mixture (1:1 vol/vol). The lipid mixture was added to a 100-mL rounded bottom flask, and the solvent was evaporated under reduced pressure at a temperature of 60°C by a rotary evaporator (BÜCHI, HB-140, Flawil, Germany) until a thin lipid film was deposited on the wall of the flask. The excess organic solvent was removed by leaving the flask in a desiccator under vacuum overnight. The lipid film was hydrated with 2.5 mL of the aqueous phase containing acyclovir sodium in a concentration equivalent to 6 mg mL⁻¹ acyclovir in normal saline. The use of drug in its sodium salt was to increase the drug concentration in the hydration fluids in a trial to increase the weight of the entrapped drug in the prepared niosomes. The hydration was continued for 1 hour, while the flask was kept rotating at 60°C. It was essential to prepare the vesicles at a temperature above the gel-liquid transition temperature of the nonionic surfactant; span 60 has the highest phase transition temperature of ~50°C. The niosomal suspension was further hydrated at room temperature for 2 hours in order to complete the swelling process. The hydrated niosomes were sonicated for 20 minutes in a bath type sonicator (Ultrasonics, Selecta, Barcelona, Spain). This niosomal dispersion containing both free and entrapped drug was used for in vivo study. Niosomes were separated from unentrapped drug by gel permeation chromatography. A 2.0-mL aliquot of the niosomal dispersions was eluted with normal saline on a 2×30-cm column of sephadex G-25. The niosomal fraction was diluted with the eluent to obtain a total lipid concentration of 5 mg mL⁻¹. This purified niosomal dispersion was used for in vitro study.

**Particle Size Determination**

The freshly purified niosomal dispersion was scanned and imaged using an optical microscope (Biomed, Carl Zeiss, Germany) attached to video camera (Panasonic, Japan) with a magnification power of ×40. Full measurement of the size and size distribution of the examined niosomes was performed using computer software that is locally designed and calibrated at the National Institute of Laser Enhanced Science (Cairo, Egypt).

**Determination of Entrapment Efficiency**

An aliquot of the freshly purified niosomal dispersion (5 mg lipid mL⁻¹) was diluted with 10% Triton X-100 in a ratio of 1:99 vol/vol. The detergent dissolved the niosomes and yielded a clear solution. The resultant solution was analyzed for acyclovir concentration using the described high-performance liquid chromatography (HPLC) method to calculate the amount of entrapped acyclovir. The percentage of entrapped acyclovir was calculated by applying the following equation:

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\% \text{ Entrapment} = \left( \frac{A_E}{A_I} \times 100 \right)
\]

where, \(A_E\) is the amount of entrapped drug, and \(A_I\) is the initial amount of drug in the aqueous phase.

**In Vitro Release Study**

The release of acyclovir from niosomes was studied by employing the dialysis method. The dialysis sacks (cellulose tubing, 35/100 mm flat width/length, Sigma Diagnostics (St Louis, MO) were washed several times with distilled water and left to soak in normal saline for 24 hours before use. A 3-mL sample, either of the freshly purified niosomal dispersion or of free acyclovir solution in normal saline, was transferred to the dialysis sacks. The concentration of acyclovir in each of the 2 samples was ~88 μg mL⁻¹ (determined according to the calculated entrapment efficiency of the niosomal dispersion). The sack was placed in 200 mL magnetically stirred normal saline at 37°C. Two milliliter samples were withdrawn at specified time intervals of 0.5, 1, 2, 3, 4, 5, and 6 hours and replaced by fresh medium, and drug content was determined according to the described HPLC method.