Improved Permeability of Acyclovir: Optimization of Mucoadhesive Liposomes Using the Phospholipid Vesicle-Based Permeation Assay

ELENAZ NADERKHANI, ASTRID ERBER, NATAŠA ŠKALKO-BASNET, GØRIL EIDE FLATEN

Drug Transport and Delivery Research Group, Department of Pharmacy, University of Tromsø, Tromsø 9037, Norway

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ABSTRACT: The antiviral drug acyclovir (ACV) suffers from poor solubility both in lipophilic and hydrophilic environment, leading to low and highly variable bioavailability. To overcome these limitations, this study aimed at designing mucoadhesive ACV-containing liposomes to improve its permeability. Liposomes were prepared from egg phosphatidylcholine (E-PC) and E-PC/egg phosphatidylglycerol (E-PC/E-PG) and their surfaces coated with Carbopol. All liposomal formulations were fully characterized and for the first time the phospholipid vesicle-based permeation assay (PVPA) was used for testing *in vitro* permeability of drug from mucoadhesive liposome formulations. The negatively charged E-PC/E-PG liposomes could encapsulate more ACV than neutral E-PC liposomes. Coating with Carbopol increased the entrapment in the neutral E-PC liposomes. The incorporation of ACV into liposomes exhibited significant increase in its *in vitro* permeability, compared with its aqueous solution. The neutral E-PC liposomal formulations exhibited higher ACV permeability values compared with charged E-PC/E-PG formulations. Coating with Carbopol significantly enhanced the permeability from the E-PC/E-PG liposomes, as well as sonicated E-PC liposomes, which showed the highest permeability of all tested formulations. The increased permeability was according to the formulations' mucoadhesive properties. This indicates that the PVPA is suitable to distinguish between permeability of ACV from different mucoadhesive liposome formulations developed for various routes of administration. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:661–668, 2014

Keywords: acyclovir; permeability; *in vitro* model; liposomes; artificial membrane; lipid; Carbopol; mucoadhesive; nanoparticles

INTRODUCTION

Acyclovir (ACV) is a commonly used antiviral agent despite the fact that most of its currently available dosage forms, that is, tablets, suspension, cream, fail to achieve suitable levels at target sites following oral, local, or parenteral administration.[1] Its absorption from gastrointestinal tract is slow and incomplete and oral bioavailabilities ranges from only 10% to 30%. As a consequence, higher doses are prescribed, resulting in systemic toxicity and adverse reactions.[2] The current therapy with ACV intended for local vaginal therapy is also limited to the conventional dosage forms, namely, tablet and cream. However, also in local therapy, its bioavailability is low and highly variable, which requires a higher administration frequency. In addition, topical administration of ACV, although preferable in pregnant patients, is associated with several disadvantages such as low retention at the vaginal epithelium, messiness, and poor patient compliance.[3,4]

The development of an efficient formulation of ACV is of increasing importance because, in spite of the continuous efforts to raise the awareness of the increased incidence of sexually transmitted diseases (STDs), their prevalence is increasing, resulting in an increased concern related to the limitations of currently available therapies. Among various STDs, the infections with human *Herpes simplex* virus type-1 and type-2 remain among the most common.[5] Particularly worrying are the recent findings by Avalos et al.[6] suggesting that there is the increased prevalence of women delivering an infant exposed to antiviral medication over time. The need for the assessment of the safety and effectiveness of antiviral medications during pregnancy is therefore of most importance.

Acyclovir, a class III drug according to Biopharmaceutics Classification System[2], is characterized by its hydrophobicity and low solubility both in water and in lipid bilayers. The bioavailability of such drugs suffering from limited solubility and permeability could be optimized and improved by controlling the drug carrier's properties such as nanoparticle size and surface characteristics.[7]

Particularly interesting as drug carriers are mucoadhesive nanopharmaceuticals, expected to enhance the contact time between the delivery system and absorbing membrane and maintaining a concentration gradient between the drug to be absorbed and the tissue, providing the enhanced delivery of the drugs to the underlying tissue, a prerequisite for the successful anti-infective therapy.[8] In vaginal therapy, the high drug concentrations in genital tissues are desirable, as this biological compartment is the relevant target site for ACV administered intravaginally.[9] In addition, through the use of mucoadhesive polymers such as poly(acrylic acid) derivatives and chitosan in the preparation of delivery systems, it is also possible to act on increased epithelial permeability of many poorly permeable drugs.[10]

To determine the permeability characteristics of drugs and drug formulations an efficient permeability model is required. A novel screening model for passive drug permeability, the phospholipid vesicle-based permeation assay (PVPA), was developed by us to mimic the intestinal epithelia.[11] Recently, also a modified PVPA mimicking healthy and compromised skin barrier

Abbreviations used: ACV, acyclovir; E-PC, egg phosphatidylcholine; E-PG-Na, egg phosphatidylglycerol sodium; HPLC, high-performance liquid chromatography; PB, phosphate buffer; PBS, phosphate buffer saline; PVPA, phospholipid vesicle-based permeation assay; STDs, sexually transmitted diseases.

Correspondence to: Gøril Eide Flaten (Telephone: +47-77646169; Fax: +47-77645151; E-mail: goril.flaten@uit.no)

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has been introduced.[12] The original PVPA barriers are prepared by depositing liposomes from egg phosphatidylcholine (E-PC) onto a filter support through centrifugation followed by a freeze–thaw cycling to promote fusing of liposomes and to obtain a tight barrier. The PVPA has been successfully used in permeability testing of the marketed drug substances, novel active substances, as well as drug in complex formulations, and has shown the potential to automation using a robotic system with a connected plate reader.[13–16] The functionality of the barriers has shown to be stable and the barriers retained their integrity within a pH range from 2.0 to 8.0.[17] Accordingly, the PVPA has proven to be suitable to both obtain information on segmental absorption in the gastrointestinal tract as well as have potential to work as permeability model for absorption sites with a broad range of pH in their environment. Further, as the PVPA barrier consist mainly of phosphatidylcholine, a component found in many absorption barriers, it has the potential to serve as a general model mimicking other absorption barriers.

The aim of this study was to improve permeability of ACV through developing and optimizing mucoadhesive liposome formulations. To assure liposomal retention on the mucosal site, liposomes were coated with mucoadhesive polymers (Carbopol® or chitosan, respectively). This approach was expected to prolong the residence time at the administration site and improve the release profile of incorporated ACV. As ACV can be applied through various routes of drug administration, we applied the well-established PVPA model developed in our laboratory as a tool in the optimization of liposome characteristics and drug permeability profile.

MATERIALS AND METHODS

Materials

Acetic acid (glacial) anhydrous GR, dinatriumhydrogenphosphat dihydrate, chloroform, and potassium chloride were purchased from Merck (Darmstadt, Germany). Acycloguanosine, potassium phosphate monobasic, sodium chloride, methanol CROMASOLV®, chitosan low molecular weight, and mucine from porcine stomach type II were obtained from Sigma–Aldrich (Steinheim, Germany). Lipoid egg phospholipids (egg phosphatidylcholine, 80% phosphatidylcholine), lipoid S 100 (soybean lecithin, >94% phosphatidylcholine), and lipoid E-PG-Na (egg phosphatidylglycerol sodium) were obtained from Lipoid (Ludwigshafen, Germany). Carbopol® 974P NF was purchased from Noveon Inc. (Cleveland, Ohio). Poloxamer 188, Pluronic® F68 NF Prill was the product of BASF Corporation (Florham Park, New Jersey). Dialysis membrane MWCO 12–14,000 Da was obtained from Medicell International Ltd. (London, UK).

Filter inserts (transwell, $d = 6.5$ mm) and plates were purchased from Corning Inc., (Corning, New York). The mixed cellulose ester filters (0.65 μm pore size) and the isopore filters (0.8 μm pore size) were obtained from Millipore (Billerica, Massachusetts). Whatman® nucleopore track-etch membrane filters (0.4 μm pore size) were obtained from Whatman (part of GE Healthcare, Oslo, Norway).

Quantification of ACV

Acyclovir was quantified by the reversed-phase high-performance liquid chromatography (HPLC) with a reverse-phase column C18 (5 μm; 3.9 × 150 mm^2; Waters, Milford, Massachusetts) on a Waters Photodiode Array Detector HPLC equipped with a UV detector (Waters). The mobile phase consisted of MeOH–MilliQ water (1:1, v/v), pH 2.5, and the flow rate was 0.4 mL/min. ACV was detected at a wavelength of 258 nm, and the measuring time adjusted to 7 min. The sample concentration was determined from a standard curve made by diluting known amounts of ACV in both water and methanol. To exclude interference because of similar retention time, separate measurements for lipid and polymer solutions, namely, phosphatidylcholine (lipoid S100), phosphatidylglycerol (PG), polymers (Carbopol®, chitosan) and solvents (Poloxamer 188, water, methanol) were performed. All measurements were carried out in triplicates. The calibration curve resulted in good linearity in the wide concentration range 0.5–50 μg/mL with R^2 of 0.9997 and 1.0000 in water and methanol, respectively.

Table 1. Liposome Composition

	Composition		
Formulation	ACV (mg)	E-PC (mg)	E-PG-Na (mg)
E-PC	20	200	
E-PC/E-PG	20	180	20

Preparation of ACV-Containing Liposomes

Liposomes containing ACV were prepared by the film hydration method and two compositions of lipids were used: E-PC (lipoid S-100) and E-PC/E-PG (9:1, weight ratio) (Table 1). The lipid components (200 mg, total lipid) and ACV (20 mg) were dissolved in methanol in a round bottom flask. The organic solvent was removed on a rotary evaporator under vacuum of 55 hPa at 45°C for a period of 1 h to remove all traces of the solvent. The dried lipid film was than hydrated with 10 mL of distilled water to obtain liposome dispersion. The liposomes were stored in refrigerator for at least 24 h for further use and characterization.

To achieve smaller and more uniformly distributed vesicles, the liposomal dispersions were sonicated by Sonics high-intensity ultrasonic processor (Sonics & Materials Inc., Newtown, Connecticut) for 1 min. The amplitude setting was 500 W/20 kHz processor 40%. Upon sonication, the liposomal suspensions were placed in refrigerator at 4°C overnight before further experiments were performed.

Coating of Liposomes

The ACV-containing liposomes, both nonsonicated and sonicated, were coated with polymers (chitosan and Carbopol) in the presence of unentrapped ACV. The 0.1% and 0.6% (w/v) chitosan solutions were prepared in 0.1% (v/v) glacial acetic acid. The 0.1% (w/v) Carbopol® 974P NF solution was prepared by dissolving Carbopol in phosphate-buffered saline (PBS), pH 7.4. The chitosan or Carbopol solutions (2.0 mL) were added dropwise to 2.0 mL of liposomal suspension under magnetic stirring at room temperature for 1 h. The coated liposomal suspensions were then placed in refrigerator overnight to stabilize. For all preparations, the rate of stirring was kept constant.[18]