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Liposomal formulation of a glycerolipidic prodrug for lymphatic delivery of didanosine via oral route

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Abstract

Didanosine is a polar drug with poor membrane absorption and high hepatic first pass metabolism. This study aimed at developing a lipidic formulation of a glycerolipidic prodrug of didanosine in order to improve its bioavailability. In the course of a preformulation study, the glycerolipidic prodrug of didanosine was characterized by microscopy, DSC and XRDT. In anhydrous conditions, the prodrug displayed a polymorphic behaviour similar to that of triglycerides. Then, we evaluated three types of lipidic formulations (emulsions, mixed micelles and liposomes) in order to encapsulate the prodrug. Solubilities in water – even in the presence of taurocholate micelles – but also in some oils were very low (max 244 μg/mL) as the prodrug was found to be amphiphilic (log $P = 2$). On the contrary, the prodrug was found to be perfectly incorporated in dipalmitoylphosphatidylcholine (DPPC) multilamellar liposomes up to a ratio of 1:5 (mol:mol) prodrug:DPPC as suggested by HPLC-UV and DSC experiments. Moreover, these liposomes could be freeze-dried whereas the chemical integrity of the prodrug was preserved. Then, the freeze-dried liposomal preparation could be formulated as gastro-resistant capsules to prevent didanosine from acidic degradation. Further experiments are on the way to evaluate *in vitro* the absorption of prodrug incorporated in liposomes by enterocytes.

Keywords: Glycerolipidic prodrug; DSC; XRDT; Liposomes; Didanosine

1. Introduction

Didanosine (ddI) is a reverse transcriptase inhibitor, well-known for the treatment of HIV infections. Administered orally, ddI has a poor bioavailability (20–40%) (Balimane and Sinko, 1999). This is attributed to the acidic degradation of ddI in the stomach, to its poor absorption due to the hydrophilic character of this molecule and to the hepatic first pass metabolism. The design of glycerolipidic prodrugs is a strategy which was previously proposed to enhance the oral bioavailability of certain compounds by mimicking long chain triglycerides (Porter and Charman, 1997; Charman and Porter, 1996). Physiologically, triglycerides are hydrolyzed in the small intestine to the corresponding 2-monoglyceride and fatty acids by pancreatic lipase and co-lipase. These metabolites are absorbed by the enterocytes in which they are re-esterified into triglycerides and packaged into intestinal lipoproteins, the chylomicrons. These latter are finally secreted into mesenteric lymph from where they can reach the systemic circulation via the thoracic lymph duct, thus, by-passing the liver (Lambert, 2000; Porter and Charman, 1997). Thus, we have considered the synthesis of a glycerolipidic prodrug of didanosine in order to increase enterocyte absorption and lymphatic transport and to by-pass the hepatic first pass metabolism, hence leading to increase the bioavailability of the drug. Noteworthy, ddI needs to be sequentially phosphorylated by host cell kinases to the 5′-triphosphate derivative to be active. The first phosphorylation is, however, the limiting step (Tan et al., 1999). Thus, another approach to improve ddI activity is to by-pass the first cellular phosphorylation by synthesizing a glycerolipidic prodrug which is mono-phosphorylated (ProddIP) (Fig. 1). In a previous paper (Lalanne et al., 2007), we described the synthesis of this prodrug of ddI. The purity was

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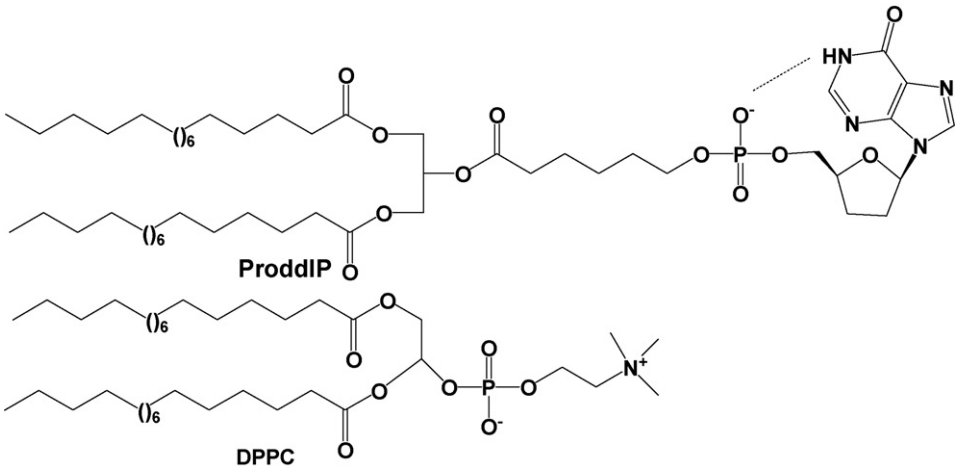

Fig. 1. ProddIP and DPPC structures.

evidenced by a validated HPLC-UV dosage method and mass spectrometry experiments.

Three types of formulations are generally suggested to enhance lymphatic absorption: emulsions, lipid mixed micelles and liposomes (Gershanik et al., 2000; Gershanik and Benita, 2000; Gursoy and Benita, 2004; Hauss et al., 1998). Because of their rich lipidic composition, these formulations generally increase chylomicron synthesis by the enterocytes. Lipophilic drugs can, indeed, gain access to the intestinal lymph through association with the products of lipid digestion and can be secreted in association with lymph lipoproteins. That is why it is also important to associate lipophilic prodrugs to lipidic compounds (long chain triglycerides or long chain phospholipids) into the formulation.

Thus, the aim of this study was to develop a lipidic formulation of this new glycerolipidic prodrug of monophosphate didanosine for oral administration. A preformulation study was first performed by polarized light microscopy, differential scanning calorimetry (DSC), and X-ray diffraction (as a function of temperature) (XRDT). Then, the ability of the three above mentioned formulations (emulsions, mixed micelles and liposomes) to incorporate the prodrug has been investigated by HPLC-UV, DSC and X-ray diffraction measurements. The stability and the protection against acidity of didanosine in the lipidic formulation chosen were also investigated.

2. Material and methods

2.1. Products

All the chemical reagents were obtained from Sigma–Aldrich (St. Louis, USA) except the ddI which was obtained from Bristol Myers Squibb (NY, USA). The solvents for the analytical section were obtained from Carlo Erba (Rodena, Italy).

Almond oil and soya oil were obtained from Cooper (Melun, France) and fish liver oil from SIRH (Fecamp, France). sodium taurocholate, sodium chloride and DPPC were obtained from Sigma (St. Louis, USA). Na_2HPO_4 and NaH_2PO_4 were obtained from VWR Prolabo (Fontenay sous Bois, France).

The prodrug (ProddIP) was synthesized as described in a previous study (Lalanne et al., 2007).

2.2. Polarized light microscopy

The prodrug and its formulation were observed by microscopy between crossed polarizers and with a λ/4 retarder in white light using a Nikon E600 Eclipse direct microscope (Champigny/Marne, France) equipped with a long focus objective (LWD 40 × 0.55; 0–2mm). NIKON Coopix 950 camera was used as a picture recorder with a resolution of 1600 × 1200 pixels.

2.3. DSC experiments

Thermal analyses were conducted by DSC, using a DSC-7 (Perkin-Elmer, St. Quentin en Yvelines, France). Samples were loaded in aluminium pans of 40 μL (pan, part no. BO14-3021, and cover, part no. BO14-3004) hermetically sealed. An empty, hermetically sealed aluminium pan was used as reference. Calibration was performed with indium (mp 156.60 °C, $\Delta H_m = 28.45$ J/g) and *n*-decane (mp −29.66 °C). Melting behaviour of the prodrug was monitored with the temperature scanning program set from 10 to 60 °C at a rate between 1 and 20 °C/min and crystallization behaviour was monitored from 60 to 10 °C at the same rate. For the thermal treatment, the prodrug was heated 15 min at 36 °C, quickly cooled to 10 °C and melting behaviour was then monitored from 10 to 60 °C at 10 °C/min.

2.4. XRDT measurements

X-ray experiments were performed with a fine-focus Cu anode source; Cu Kα (λ = 1.54 A) radiation was selected and line focused by a multilayer mirror and collimated by slits. A microcalorimeter cell, MICROCALIX (Keller et al., 1998; Ollivon et al., 2006), which allowed simultaneous thermal and X-ray measurements, was used as sample holder. Samples were loaded in Lindemann glass capillaries (diameter 1.5 mm) and