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Oral 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycero-3-cidofovir targets the lung and is effective against a lethal respiratory challenge with ectromelia virus in mice☆

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Abstract

Hexadecyloxypropyl-cidofovir (HDP-CDV) has been shown to be orally active against lethal infection with orthopoxviruses including, mousepox, cowpox, vaccinia and rabbitpox. The alkoxyalkyl group provides oral absorption and reduces greatly the amount of drug reaching the kidney, the site of CDV's dose limiting toxicity. However, the amount of HDP-CDV detected in lung, an important site of early poxvirus replication, is low and the reduction of viral titers in surviving animals is reduced moderately compared with the liver where poxvirus titers are virtually undetectable. We synthesized a novel glycerol ester of CDV, 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycero-3-CDV (ODBG-CDV), and compared its oral pharmacokinetics with that of HDP-CDV. Surprisingly, ODBG-CDV levels in lung are much higher and liver levels are reduced, suggesting that the compound is transported in small intestinal lymph instead the portal vein. ODBG-CDV has excellent in vitro activity in cells infected with ectromelia virus (ECTV). In mice infected with a lethal aerosol or intranasal challenge of ECTV, HDP-CDV and ODBG-CDV are equally effective in preventing death from disease. Other drugs esterified to 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol or 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol-3-phosphate may provide lung targeting for treatment of microbial or neoplastic diseases while reducing first pass removal by the liver during oral absorption.

Keywords: Ectromelia virus; Cidofovir; Prodrugs; Lung targeting

1. Introduction

It has been previously reported that alkoxyalkyl analogs of acyclic nucleoside phosphonates like hexadecyloxypropyl-cidofovir (HDP-CDV) are orally bioavailable and active in lethal orthopoxvirus challenge models (Buller et al., 2004; Quenelle et al., 2004). However, oral pharmacokinetics with radiolabeled HDP-CDV (Ciesla et al., 2003) show low levels of drug and metabolites in the lung, an important site of early poxvirus replication. We reported previously that 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycero-cidofovir (ODBG-CDV; Fig. 1) was active against cowpox and vaccinia virus strains with 50% effective concentration values (EC_{50}) ranging from 0.09 to 0.4 μM (Wan et al., 2005). ODBG-CDV was also highly active against HCMV, ganciclovir-resistant and phosphonformate-resistant isolates of HCMV, HSV-1, HSV-2, VZV, EBV, HHV-6A, HHV-6B and HHV-8 with EC_{50} values in the nanomolar range (Williams-Aziz et al., 2005). In vaccinia infection in organotypic epithelial raft cultures of primary human keratinocytes, ODBG-CDV was the most active analog of CDV with an EC_{90} value <0.04 μM (Lebeau et al., 2006).

We prepared ODBG-[2-^{14}C]-CDV to assess oral and parenteral pharmacokinetics. The compound was given orally and intraperitoneally to mice and tissue and plasma levels of radiolabeled drug and metabolites were measured at various times up to 72 h. Relative oral bioavailability was determined from the plasma area under curve (AUC) obtained with oral versus intraperitoneal administration. Tissue distribution of drug and metabolites was assessed in liver, kidney, and lung. Surprisingly,

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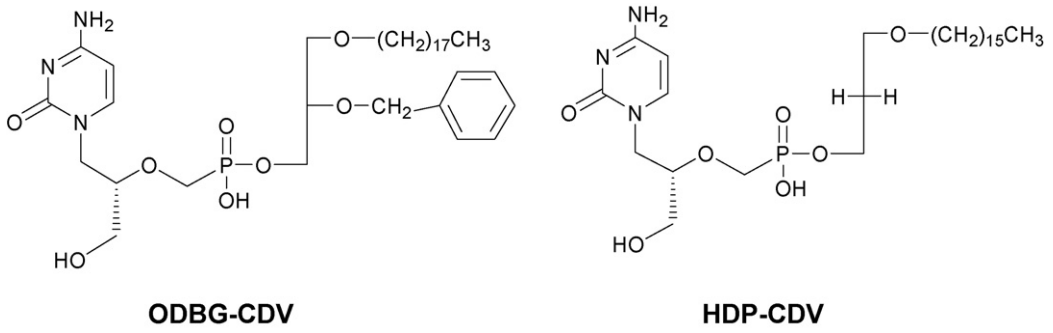

Fig. 1. Structures of 1-*O*-octadecyl-2-*O*-benzyl-glycero-cidofovir and hexadecyloxypropyl-cidofovir.

oral ODBG-[2-^{14}C]-CDV produced very high levels of drug and metabolites in lung compared with oral HDP-[2-^{14}C]CDV, indicating that oral ODBG-[2-^{14}C]-CDV targets the lung in mice. ODBG-CDV and HDP-CDV were evaluated orally in aerosol and intranasal ectromelia virus (ECTV) lethal challenge models. Both compounds were highly effective in preventing death from disease.

2. Methods

2.1. Chemistry

1-*O*-Octadecyl-2-*O*-benzyl-*sn*-glycerol was purchased from Bachem, Torrance, CA. 1-*O*-Octadecyl-2-*O*-benzyl-*sn*-glycero-3-cidofovir was synthesized as previously described (Wan et al., 2005). Briefly, anhydrous cCDV (1 equiv.), 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glycerol (2 equiv.), and triphenylphosphine (2 equiv.) were dissolved/suspended in anhydrous *N*,*N*-DMF (6.5 ml/mmol of cCDV), and stirred vigorously under a nitrogen atmosphere. Diisopropyl azadicarboxylate (DIAD, 2 equiv.) was then added in three equal portions over 15 min before the mixture was allowed to stir overnight. The solvent was then evaporated under vacuum and the residue purified by column chromatography with silica gel and recrystallized from *p*-dioxane. The cyclic CDV ring was opened with base (Wan et al., 2005). Using this method, ODBG-[2-^{14}C]-CDV was prepared by Moravek Biochemicals, Inc. (Brea, CA). The structures of HDP-CDV and ODBG-CDV are shown in Fig. 1.

2.2. Animal pharmacokinetic studies

Female Swiss-Webster mice weighing approximately 25 g received a single dose of 10 mg/kg ODBG-[2-^{14}C]-CDV (specific activity, 53 mCi/mmol) in sterile 0.9% saline either by oral gavage or by intraperitoneal injection. Three animals were sacrificed at 1, 3, 6, 12, 24, 48, and 72 h and blood and tissue samples were obtained. Fifty microlitres of plasma was added to 10 ml of Ecolite cocktail and analyzed for drug and metabolite content by a scintillation counting. The kidney, lung and liver were also obtained from each mouse, washed with 0.9% saline, and weighed. The tissue was treated with 3 ml TS-2 tissue solubilizer and 0.5 ml of water and placed in a 50 °C water bath for 36–48 h. Glacial acetic acid was added to each vial to neutralize the TS-2 and Flo-Scint IV was added before counting. Plasma pharmacokinetic data was calculated as reported previously (Ciesla et al., 2003).

2.3. HPLC analysis of metabolites

2.3.1. Lung and liver tissue

Twenty percent homogenates of liver and lung tissue were made in ultrapure distilled water saline and aliquots were reserved frozen at −70 °C until analysis. A portion of the liver and lung homogenates was frozen and thawed two times in an isopropanol/dry ice bath and then sonicated on ice in a bath sonicator for 5 min. Trichloroacetic acid was added to a 7% final concentration and centrifuged at 4 °C for 10 min at 1000 rpm. The supernatant was removed and aliquots counted in a scintillation counter. An aliquot representing approximately 10,000 DPM was analyzed by HPLC (System Gold, Beckman Coulter, Fullerton, CA). The samples were injected into a Partisil 10 SAX column (Alltech, Deerfield, IL), 4.6 cm × 15 cm, and a SAX guard column. Metabolites were eluted at a flow rate of 1 ml/min using a potassium phosphate buffer gradient of 20–700 mM, pH 5.8, beginning at 9 min for 20 min followed by a 5 min terminal hold. One minute fractions were collected and Ultima Flo scintillation fluid added and their content of radioactivity was determined by liquid scintillation. The identity of the radioactive peaks was compared with the retention time of pure standards of CDV, CDV-monophosphate (CDVp) and CDV-diphosphate (CDVpp). CDVp and CDVpp were obtained from TriLink Biotechnologies Inc. (San Diego, CA).

2.4. Ectromelia virus studies

2.4.1. Plaque reduction assay

BSC-1 cells were plated in wells of a 24 well cluster plate. Each monolayer was infected with ∼75 plaque forming units (PFU) of indicator virus in 0.1 ml of DMEM +2% fetal clone II for 60 min at 37 °C. Media was removed by aspiration and standard virus overlay media containing no drug or the test drug at concentrations ranging from 0.05 to 50 μM was added. The plates were incubated at 37 °C for 3–4 days and monolayers were