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Effect of nonalcoholic steatohepatitis on renal filtration and secretion of adefovir

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ABSTRACT

Background and aims: Adefovir, an acyclic nucleotide reverse transcriptase inhibitor used to treat hepatitis B viral infection, is primarily eliminated renally through cooperation of glomerular filtration with active tubular transport. Nonalcoholic steatohepatitis is a variable in drug disposition, yet the impact on renal transport processes has yet to be fully understood. The goal of this study was to determine the effect of nonalcoholic steatohepatitis on the pharmacokinetics of adefovir in rats given a control or methionine and choline deficient diet to induce nonalcoholic steatohepatitis.
Methods: Animals received a bolus dose of 7 mg/kg (35 μCi/kg) [^{3}H] adefovir with consequent measurement of plasma and urine concentrations. Inulin clearance was used to determine glomerular filtration rate.
Results: Methionine and choline deficient diet-induced nonalcoholic steatohepatitis prolonged the elimination half-life of adefovir. This observation occurred in conjunction with reduced distribution volume and hepatic levels of adefovir. Notably, despite these changes, renal clearance and overall clearance were not changed, despite markedly reduced glomerular filtration rate in nonalcoholic steatohepatitis. Alteration of glomerular filtration rate was fully compensated for by a significant increase in tubular secretion of adefovir. Analysis of renal transporters confirmed transcriptional up-regulation of Mrp4, the major transporter for adefovir tubular secretion.
Conclusions: This study demonstrates changes to glomerular filtration and tubular secretion that alter pharmacokinetics of adefovir in nonalcoholic steatohepatitis. Nonalcoholic steatohepatitis-induced changes in renal drug elimination processes could have major implications in variable drug response and the potential for toxicity.

1. Introduction

Adefovir, an acyclic nucleotide reverse transcriptase inhibitor, is approved for the therapy of chronic hepatitis B, in adults with permanently increased serum alanine transaminase (ALT) activity, and histological evidence of active liver inflammation and fibrosis [1,2]. In addition to significant clinical efficacy against HBV infection, adefovir can be used during decompensated liver disease because it is dependent upon the renal processes of glomerular filtration and active tubular secretion for elimination [3,4]. However, it is now clear that liver impairment is associated with changes in glomerular filtration [5], which may create predisposition for unpredictable pharmacokinetic behavior of compounds, and sensitize the liver for further impairment. Within the course of adefovir therapy, such problems may be anticipated because patients with decompensated liver disease have higher incidence of increased serum levels of creatinine, a marker of glomerular filtration [6,7].

Nonalcoholic fatty liver disease (NAFLD) and its late stage, nonalcoholic steatohepatitis (NASH), contribute to increased sensitization of the liver to noxious stimuli [8]. Although Wang et al. [9] assert in their recent systemic review that chronic HBV infection

Abbreviations: ABC, ATP-binding cassette family; *ABCC4*, ATP-binding cassette family, sub-family C, gene 4; ADR, adverse drug reaction; ERK, extracellular signal-regulated kinase; GFR, glomerular filtration rate; MCD, methionine and choline deficient diet; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

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protects against fatty liver, it has also been suggested that metabolic syndrome (largely associated with NAFLD) accelerates the progression of NAFLD to NASH in patients with HBV [10]. This suggests a possible overlap between patients with NASH and those taking adefovir chronically for HBV infection. This may be problematic, as NASH is also associated with misregulation of excretory mechanisms for many drugs. The remodeling of hepatic transport processes in NASH has been well characterized over the last several years, both in the human disease and in a variety of rodent models [11,12]. While several OATP uptake transporters are downregulated at basolateral membranes of hepatocytes [13], principal efflux transporters for anionic drugs are altered both at the basolateral (MRP3/4) and canalicular (MRP2/BCRP) membranes of hepatocytes to favor plasma retention for many substrates [11,14–16]. These alterations are also documented in a variety of genetic and dietary models of NASH [12], many of which also exhibit regulatory changes in prominent renal transporters [17]. To date, very little is known regarding the changes in renal transporter expression in human NASH, and the functional consequence to the pharmacokinetics and potential for toxicity of many drugs.

It is well known that renal excretion of adefovir is mediated by both glomerular filtration and active transporter-mediated tubular secretion; although, the exact contribution of both processes to renal clearance of adefovir is not evident [18,19]. Two major transporters involved in adefovir secretion are organic anion transporter 1 (OAT1; *SLC22A6 gene*) and organic anion transporter 3 (OAT3; *SLC22A8 gene*) [20,21] on the basolateral membranes of renal proximal tubular cells where they act in the uptake of adefovir. The efflux transporter responsible for apical transport is multidrug resistance protein 4 (MRP4; *ABCC4 gene*) [19,22].

This purpose of this study was to determine the effect of NASH on glomerular filtration and tubular secretion of adefovir, and to identify mechanistic changes in renal drug transporters (Mrp4, Oat1, Oat3). The MCD diet was utilized to induce NASH because of its broad use, its similarity in modeling the pharmacokinetic changes seen in human NASH, and the histologic features that closely resemble the human pathology [12,23].

2. Materials and methods

2.1. Chemicals

[^{3}H] Adefovir (11.9 Ci/mmol) and [^{14}C] Inulin methoxy (6.2 mCi/g) (Moravek Biochemicals Inc., Brea, CA), Inulin, adefovir, and urethane (Sigma–Aldrich, St. Louis, MO), 20% mannitol (Baxter Healthcare Corporation, Deerfield, IL), Ultima GOLD and Solvable (Perkin Elmer, Waltham, MA). Radiolabeled adefovir was used to facilitate detection throughout the course of the study, and because scintillation counting would not be complicated by metabolite presence. The rate of decomposition of radiolabeled compounds guaranteed by supplier is approximately 1%/month for the first six months after purification when stored at −20 °C. Both radiolabeled compounds were therefore used within the 1 month after delivery from supplier.

2.2. Animals

Twelve male Sprague–Dawley rats weighing 200 g were obtained from Harlan (Indianapolis, IN). The animals were housed in AAALAC approved facilities with a standard 12 h light/dark schedule. Housing and experimental procedures were in accordance with National Institutes of Health guidelines and were approved by the University of Arizona Institutional Animal Care and Use Committee, maintaining a temperature and humidity range of 20–26 °C and 30–70%, respectively. Rats were fed a methionine and choline sufficient (control) or a methionine and choline deficient (MCD) diet (Dyets, Inc., Bethlehem, PA) for 8 weeks.

2.3. Adefovir in vivo clearance study

All rats were under urethane anesthesia (5 mg/kg i.p.), with body temperature maintained at 37 °C by heat platform for the duration of the study. The rats were cannulated with polyethylene tubes in the jugular vein for drug administration and infusion, and jugular artery for blood collection. The trachea was cannulated to keep the airways clear, and the urinary bladder was cannulated for urine collection. The rats received single-dose bolus of adefovir (7 mg/kg; 35 μCi/kg) and inulin in a loading dose of (10 mg/kg; 15 μCi/kg) followed by constant-rate infusion (Sage Instruments Syringe pump Model 351) of 4% mannitol delivering 20 mg/kg (15 μCi/kg) of inulin per hour at a rate of 2 ml/h until the end of the study. Mannitol (4% saline solution) was used to obtain a sufficient and constant urine flow rate. Urine was collected in preweighed tubes at 30 min intervals for 240 min throughout the experiment. Blood samples were collected at 4, 10, 30, 60, 75 (midpoint for GFR calculation), 120, 180 and 240 min. Plasma samples were obtained by centrifugation of the blood samples at 3000*g* for 10 min. The volumes of urine were measured gravimetrically, with specific gravity assumed to be 1.0. All plasma and urine samples were stored at −80 °C until analysis.

2.4. Liquid scintillation counting

To determine radioactivity in samples, 15 μl plasma or urine was diluted in 5 ml UltimaGold scintillation cocktail. A 50 mg sample of liver or kidney was incubated with 500 μl Solvable for 4 h at 60 °C and subsequently mixed with 5 ml UltimaGold. All samples were counted for disintegrations per minute (dpm) of ^{3}H and ^{14}C using on a Beckman LS6500 IC scintillation counter, using the dual-label program (10 min) and converted to concentrations using the specific activities and molar masses of adefovir and inulin.

2.5. Western blot

Whole cell lysate preparations of rodent kidney were prepared from 300 mg of tissue homogenized in Nonidet-P40 Buffer (20 mM Tris–HCl, 137 mM NaCl, 10% glycerol, 1% nonidet P-40, 2 mM EDTA with 1 Protease Inhibitor Cocktail Tablet (Roche, Indianapolis, IN)) on ice. Homogenized tissue was agitated at 4 °C for 2 h, centrifuged at 10,000*g* for 30 min, and the supernatant was transferred to a clean collection tube. Protein concentrations were determined using the Pierce BCA Protein Quantitation Assay (Thermo Fisher Scientific, Rockford, IL) per the manufacturer's recommendations and stored at −80 °C until further analysis. Whole-cell lysate kidney proteins (50 μg/well) were separated by SDS–polyacrylamide gel electrophoresis on 7.5% gels and transferred to polyvinyl difluoride membranes. The following antibodies were used: Oat1 (rabbit polyclonal, 1:500; Abcam, Inc., Cambridge, MA), Oat3 (goat polyclonal, 1:1,000 Santa Cruz Biotechnology Inc., Dallas, TX), Mrp4 (rat monoclonal, 1:800; Abcam, Inc.), and Erk2 (rabbit polyclonal, 1:1,000; Santa Cruz Biotechnology Inc.). The following horseradish peroxidase-conjugated secondary antibodies were used: anti-rat (1:20,000), antirabbit (1:10,000) and anti-goat (1:10,000Santa Cruz Biotechnology). Relative protein expression was quantified using image processing and analysis with ImageJ software (National Institute of Health, Bethesda, MD) and normalized to Erk2.