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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.

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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

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 down-regulated at basolateral membranes of hepatocytes [13], principal efflux transporters for anionic drugs are altered both at the baso-lateral (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

[³H] Adefovir (11.9 Ci/mmol) and [¹⁴C] 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 pre-weighed 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 3000g 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 ³H and ¹⁴C 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 10,000g 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 (1:10,000 Santa 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.

2.6. Branched DNA assay

Total RNA was isolated from rodent kidney tissue using RNAzol B reagent (Tel-Test Inc., Friendswood, TX) per manufactureŕs recommendation, and quantified using NanoDrop (Thermo Fisher Scientific Inc.). Specific oligonucleotide probes for rat *Abcc4*, *Slc22a6*, *Slc22a8* were diluted in lysis buffer supplied by the Quantigene HV Signal Amplifications Kit (Genospectra, Fremont, CA). Substrate solution, lysis buffer, capture hybridization buffer, amplifier, and label probe buffer used in the analysis were all obtained from the Quantigene Discovery Kit (Genospectra). The assay was performed in 96-well format with 10 μg of total RNA added to the capture hybridization buffer and 50 μl of diluted probe set. The total RNA was then allowed to hybridize to the probe set overnight at 53 °C. Hybridization steps were performed per the manufacturer's protocol on the following day. Luminiscence of the samples was measured with a Quantiplex 320 bDNA luminometer interfaced with Quantiplex Data Management Software (version 5.02).

2.7. Kidney histology

Paraffin-embedded kidney and liver sections were stained with hematoxylin and eosin at the University of Arizona Histology Core. Kidney sections, not blinded, were evaluated and scored for pathologic renal changes by Research Pathology Services, Rutgers University. Renal tubule endpoints included tubular degeneration, necrosis and regeneration, epithelial cell loss and pigment accumulation. Scoring criteria are as follows: 0, no noteworthy changes; 1, minimal (10% affected); 2, mild (10–25% affected); 3, moderate (25–40% affected); 4, marked (40–50% affected); 5, severe (>50% affected). Endpoints of glomerular damage included (1) increased glomerular cellularity, (2) increased inflammatory cells (lymphocytes, macrophages and/or neutrophils), (3) thickening of the glomerular basement membrane, (4) thickening of the Bowman's capsule, and (5) reduced glomerular size (atrophy).

2.8. Data analysis

Analysis of adefovir pharmacokinetics was performed by standard non-compartmental approach using Kinetica software (Thermo Fisher Scientific Inc.). Non-compartmental analysis was used to describe the disposition of adefovir during 240 min. The renal clearance of inulin was calculated by dividing the renal excretion rate in 60–90 min time point by the C_{ss} determined in plasma in the midpoint of this time collection period (75 min). Maximum observed serum concentrations (C_{max}) of adefovir were estimated for each animal directly from the serum concentration time data. The time of the maximum concentration (T_{max}) was defined as the time of the first occurrence of C_{max} (i.e. coincident with the initial blood sample). The elimination rate constant (K_{el}) was estimated by subjecting the plasma concentrations in the terminal phase to linear regression analysis. The terminal elimination phase half-life ($T_{1/2\beta}$) was calculated by dividing ln 2 by K_{el}. Area under the plasma concentration–time curve ($AUC_{0-Tlast}$) from time 0 to T_{last} was estimated according to the log-linear trapezoidal rule where T_{last} was the last quantified concentration. Total AUC (AUC_{Tot}) was the sum of $AUC_{0-Tlast}$ and AUC_{Extra} calculated as the last measured concentration divided by K_{el}. %AUC_{Extra}, the marker of sufficient duration of evaluation, stands for % of AUC_{Extra} from AUC_{Tot}. The total clearance (CL_{tot}) was calculated as the ratio of applied dose to AUC_{Tot}. The renal (CL_R) clearance was calculated by division of the amount of adefovir excreted in urine by its $AUC_{0-Tlast}$. The apparent volume of distribution (V_d) was calculated as $V_d = CL_{Tot}/K_{el}$. GFR was represented as inulin clearance. A 60 min constant infusion of inulin after a single dose bolus of 10 mg/kg of inulin was found to result in a steady-state concentration [19,24].

The renal clearance of inulin was calculated by dividing the renal excretion rate in 60–90 min time point by the C_{ss} determined in the midpoint plasma (75 min). Tubular secretion of adefovir was calculated by subtraction of GFR from CL_R.

Experiments were carried out on 5 animals per group. All experimental data are expressed as mean ± SD. Differences between experimental and control values were assessed by unpaired t-test using GraphPad Prism 6.0 software (GraphPad Software, San Diego, California). A value of $p < 0.05$ was considered statistically significant.

3. Results

To evaluate pharmacokinetics of adefovir, a single intravenous bolus dose was administered and blood and urine were collected over 240 min. NASH had no influence on the plasma concentrations of adefovir as indicated by the plasma concentration–time profile (Fig. 1) and the unchanged AUC_{tot}, the volume of distribution, and CL_{tot} (Table 1). On the other hand, the terminal half-life of adefovir was prolonged in NASH animals, which indicates alteration in the elimination processes of the agent. Induction of NASH by MCD feeding did not change overall urine production (Fig. 1). A majority of the administered dose was eliminated in the urine for both control and NASH rats and cumulative urinary excretion of the drug was not significantly changed (Fig. 1). A significant increase in the urinary concentration ($p < 0.001$) and absolute excretion of adefovir ($p = 0.007$) was observed at 60 min in NASH (Fig. 1). The glomerular filtration rate as measured by inulin clearance was markedly reduced in NASH (Table 1). This effect was not, however, associated with reduced renal clearance of the drug because NASH induced tubular secretion clearance of adefovir, as calculated from the difference between renal clearance of the agent and glomerular filtration rate ($p < 0.001$).

Concentration of [³H] adefovir was also determined in the liver and kidney tissue of control and NASH animals at the end of clearance study (Fig. 2). The concentration of adefovir was significantly decreased in NASH livers compared to the control ($p = 0.0039$). The concentration of adefovir was not significantly changed in the kidney ($p = 0.198$).

To determine the potential influence of NASH on the renal expression of three transporters important for uptake and efflux of adefovir, the levels of mRNA expression of vital transporter proteins (Oat1, Oat3, Mrp4) were analyzed using branched chain DNA analysis (Fig. 3). The mRNA expression of Oat1 was not changed. The mRNA expression of Oat3 was upregulated in the NASH group ($p = 0.005$), as was Mrp4 ($p = 0.018$; Fig. 3).

The protein expression of these transporters was analyzed in whole cell kidney fraction and is shown in Fig. 4. Protein expression of Mrp4 was upregulated ($p = 0.028$) in the NASH group, which is consistent with the mRNA analysis. Protein expression of Oat1 ($p = 0.843$) was not changed, nor was Oat3 ($p = 0.125$) despite the mRNA expression increase (Fig. 4).

The effect of NASH on renal morphology was determined via histological examination. All MCD diet rats had pathologic changes while control rats had none. The severity of kidney pathology is summarized in Table 2, and the kidney histology of control and NASH rats is shown in Fig. 5. The overall score was considered the best indicator to renal injury, and was markedly increased in MCD animals as a consequence of changes seen in all measured parameters – regeneration, degeneration, necrosis, epithelial cell loss, and pigment accumulation. Tubular epithelial regeneration was characterized by tubules with prominent lumens lined by plump epithelial cells with hyperchromatic nuclei and occasional mitoses. Degenerate cells were swollen, with hypereosinophilic, variably vacuolated cytoplasm. Necrotic tubules had pyknotic or
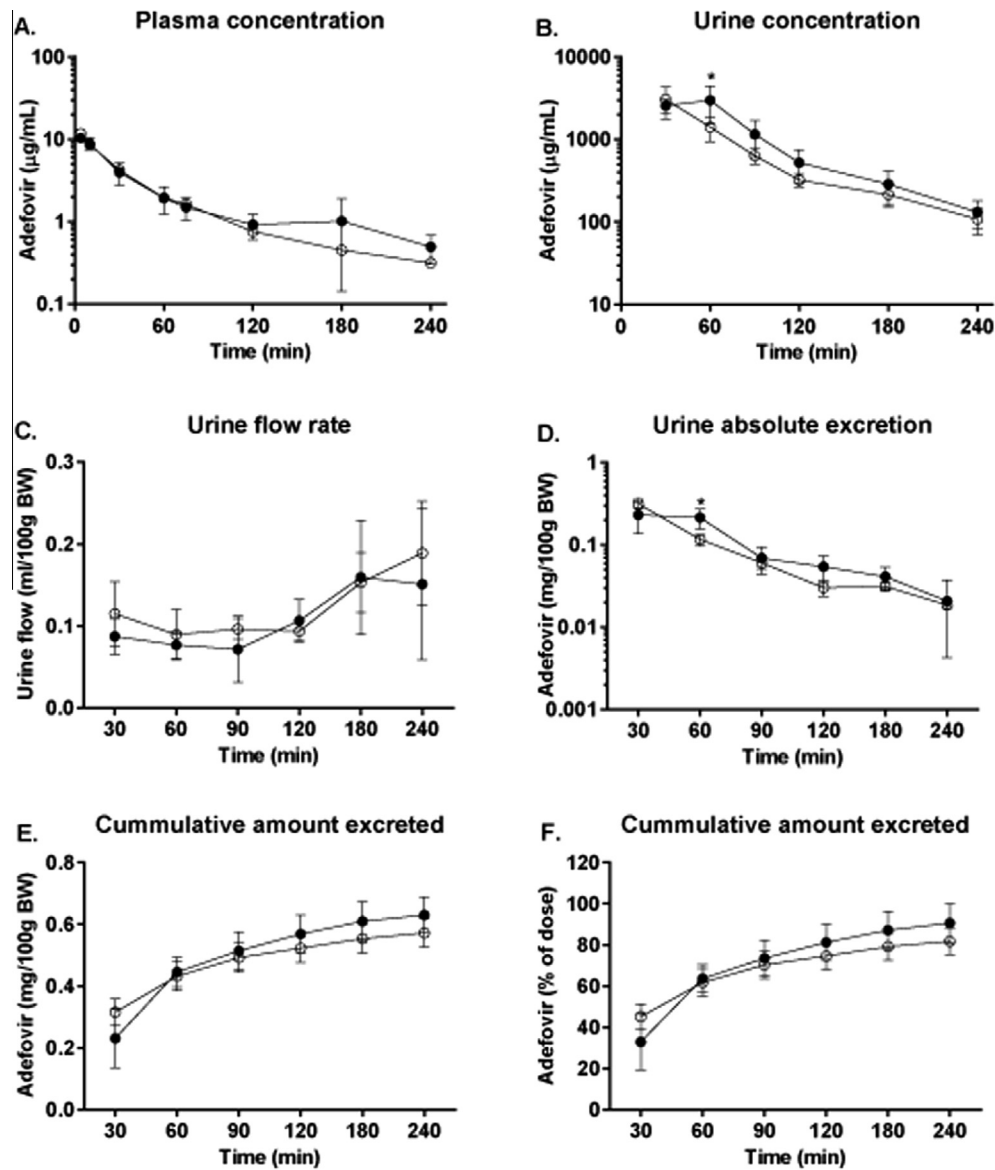
Fig. 1. Plasma concentration and urinary excretion of adefovir in control and NASH rats. The plasma concentration (A), urinary concentration (B), urinary excretion (% of total dose; C), absolute excretion (D) and cumulative excretion (E) of [³H] adefovir were determined during an *in vivo* clearance study after a single-dose bolus in control (○) and NASH (●) rat groups. Each point represents mean ± SD (*n* = 5). *, *p* < 0.05 statistically significant difference.

Table 1
Pharmacokinetic parameters of adefovir in control and NASH rats.

	Control	NASH	*p*-value
Cl$_{tot}$ (ml/min × 100 g bw)	1.4 ± 0.18	1.2 ± 0.29	0.182
AUC$_{total}$ (mg/l/min)	502 ± 67	606 ± 125	0.103
%AUC$_{extra}$ (%)	9.2 ± 2.72	20.7 ± 10.4*	0.026
CL$_R$ (ml/min × 100 g bw)	1.3 ± 0.25	1.4 ± 0.33	0.567
GFR (ml/min × 100 g bw)	0.8 ± 0.20	0.4 ± 0.28*	0.0173
Tubular secretion (ml/min × 100 bw)	0.4 ± 0.16	0.9 ± 0.15*	0.0002
V$_d$ (L/100 g bw)	0.21 ± 0.07	0.24 ± 0.07	0.456
C$_{max}$ (μmol)	44.4 ± 9.10	39.8 ± 1.30	0.2484
T$_{1/2β}$ (min)	100 ± 22.3	140 ± 20.0*	0.0084

Values are show as Mean ± SD (*n* = 5). Significantly different from control value (*p* < 0.05). NASH, nonalcoholic steatohepatitis.

karyorhectic nuclei, hypereosinophilic cytoplasm and intraluminal (sloughed) epithelial cells. Epithelial cell loss was indicated by reduced numbers of epithelial cells lining tubules. Accumulation of golden-brown pigment (lipofuscin) was present in tubules and interstitial spaces of the cortex. There were no noteworthy glomerular changes (Fig. 5).

4. Discussion

In recent years the prevalence of NAFLD has been steadily increasing, making it the most common liver disease worldwide, and in Western countries, NAFLD incidence is correlated to lifestyle habits [25]. Recent advances indicate that the severe form of NAFLD, NASH, can cause significant alterations in liver transporter expression and xenobiotic pharmacokinetics [14,16,26–29], and can therefore lead to increased susceptibility for adverse drug reactions (ADRs) [13,30–32]. Moreover, recent studies demonstrate that various animal models of NASH also cause significant alterations in the expression of membrane transporters in the kidneys, although no one has demonstrated the functional consequence of these alterations [26,33]. Herein, we found that upregulation of renal efflux transporters involved in luminal secretion may compensate for the decreased GFR frequently observed in liver injury,
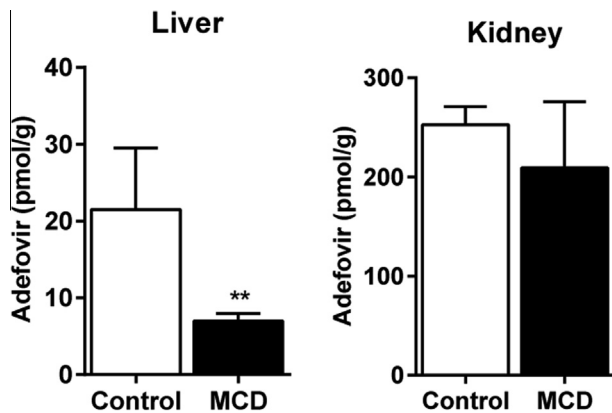
Fig. 2. Concentration of adefovir in liver and kidney tissue in control and NASH rats. The liver (A) and kidney (B) tissue concentrations of [^{3}H] adefovir were determined in control (open column) and NASH (closed column) rat groups. Bar represents mean ± SD (n = 5). **, $p < 0.01$ statistically significant difference using Student's T-test.

by supporting the renal secretion of xenobiotics and endogenous metabolites into the urine [17].

We therefore evaluated the influence of modeled NASH on the pharmacokinetics of adefovir, a drug eliminated primarily by kidneys. The plasma concentrations of adefovir showed no significant differences between the normal and NASH groups. Adefovir was rapidly cleared from plasma when administered as an i.v. single bolus dose, with the initial half-life of approximately 15 min, which corresponds with previous studies in mice and rats [34,35] and a terminal elimination half-life of approximately 2 h. While the total clearance of adefovir was not changed in NASH, the contribution of renal excretory pathways to its elimination was markedly altered. The glomerular filtration in the NASH group decreased by about 50%, but this was counterbalanced by a net tubular secretion increase compared to control group, mediated via induction of Mrp4, the major efflux transporter for adefovir. As a consequence, total clearance of adefovir was not changed, but its concentration profiles in urine were different. These data suggest that Mrp4 may be a rate-limiting factor for adefovir tubular secretion, as its induction was able to rescue the potentially decreased renal elimination stemming from GFR changes. In support of this conclusion, increased accumulation of adefovir was recently described in human proximal tubular cells when MRP4 transporter was downregulated by hypoxia [36].

We observed a non-significant decrease in urinary adefovir concentration at the first time point (30 min) and consecutive increases in urinary concentration at the second time point (60 min) in the NASH group, which changed the shape of the urinary excretion over time curve. This finding corresponds with the changes of GFR and tubular secretion of adefovir based on the upregulation of Mrp4 in the kidney. It suggests that decreased GFR in NASH rats may slow the filtration of adefovir and delay its initial appearance in urine. However, upregulated Mrp4 transporter protein resulted in increased tubular excretion of the drug, and subsequently contributed to increased liver adefovir. The change of the elimination curve shows how NASH influences the total renal clearance and how this disease could change both excretory mechanisms in different ways. Moreover, changes in GFR and tubular secretion during NASH may result in substantial variability in the elimination of specific drugs which, unlike adefovir, rely exclusively on one of those elimination processes (e.g. pencillins by tubular secretion or aminoglycosides by GFR).

To establish the GFR we used the standard method determined by inulin clearance, which is more reliable than the endogenous creatinine clearance and is recommended as an easier and more reproducible assay to perform. Our estimated values of GFR in the control group are consistent with previous findings of other researchers. The mean GFR in our control group was 0.84 ± 0.20 ml/min $\times$ 100 g bw, while Wistar rats are reported to have 0.86 ± 0.19 ml/min $\times$ 100 g bw using the FITC-inulin, or 0.84 ± 0.13 ml/min $\times$ 100 g bw using the [^{14}C]inulin [37]. Though we used Sprague–Dawley rats in our study, total kidney GFR is not different between Sprague–Dawley and Wistar strains [38]. Before this study, it was unknown that the MCD diet would affect GFR in rats. It is known that reduction of GFR can be caused either by decreased renal perfusion and/or damage to glomerular membranes. A previous study [17] observed mild mesangial expansion of glomeruli in MCD rats but the effect on GFR was not measured. The MCD cohort in this study does not demonstrate this same pathological manifestation, although a decreased GFR was clarified with a reduced V_d and prolonged $T_{1/2\beta}$ of adefovir. Lack of microscopic alterations in the glomeruli of our NASH animals is insufficient to rule out any source of glomerular dysfunction, especially given the previously observed glomerular pathology. Detection of subcellular impairment of glomeruli is beyond resolution of light microscopy and cannot be excluded because the mechanisms responsible for the association between NASH and kidney injury are not fully understood. There is evidence suggesting that NAFLD, and especially NASH, could be involved in the pathogenesis of kidney injury through pro-inflammatory cytokines (TNF-α, IL-6, TGF-β), reactive oxygen species and other pathogenic mediators [39–41]. Targher et al. [5] found that human patients with NASH diagnosed by liver biopsy had lower eGFR (estimated GFR based
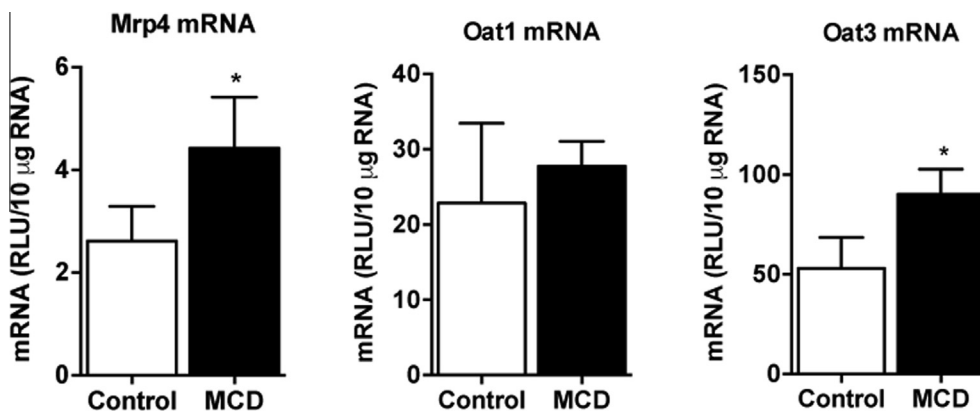

Fig. 3. Kidney mRNA expression of drug transporter genes in control and NASH rats. Renal mRNA expression of Mrp4, Oat1, and Oat3 mRNA in control (open column) and NASH (closed column) rat groups. Bar represents mean ± SD (n = 5). *, $p < 0.05$; **, $p < 0.01$ statistically significant difference using Student's T-test.
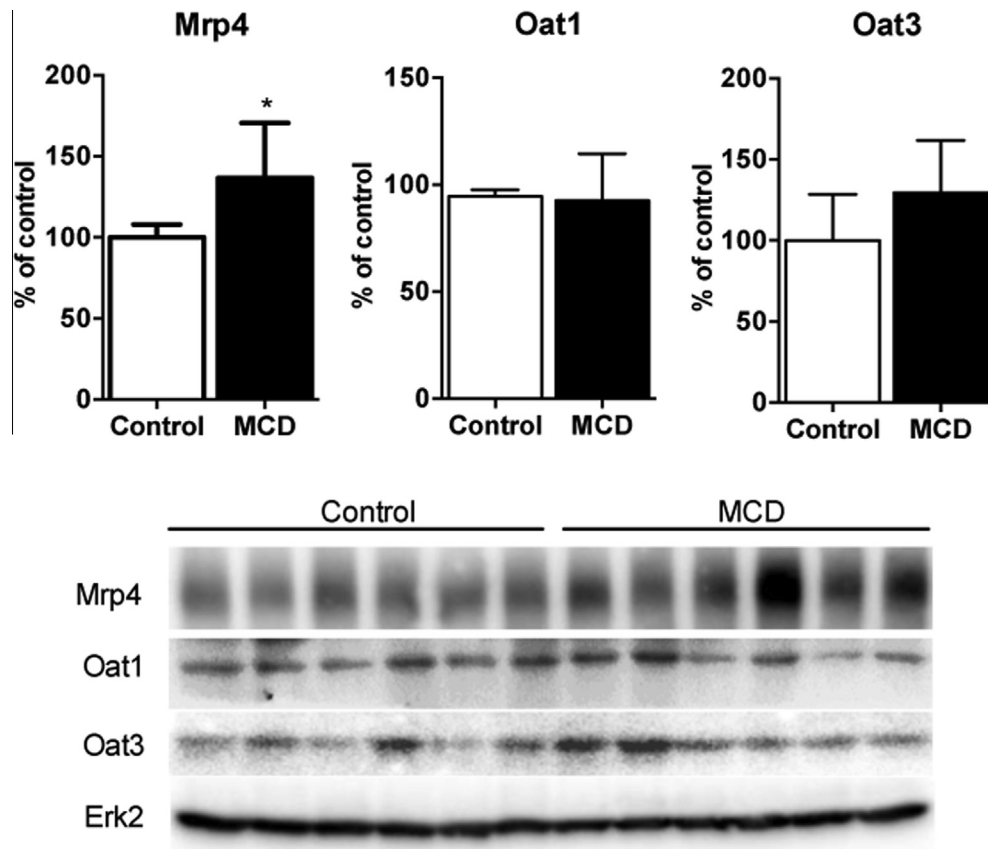
Fig. 4. Western blot analysis of protein expression of drug transporter proteins in the kidney. Renal protein expression of Mrp4, Oat1, and Oat3 normalized to Erk2 (% of control) are depicted for control (open column) and NASH (closed column) rat groups. Bar represents mean ± SD ($n = 5$). *, $p < 0.05$ statistically significant difference using Student's *T*-test.

Table 2

Kidney pathology severity scores in control and NASH rats. Semiquantitative values represent the median (range) of $N = 5$ animals and were rounded to next whole number (median of 1.5 is reported as 2). Scoring key: 0, none; 1, minimal (<10% affected); 2, mild (10–25% affected); 3, moderate (25–40% affected); 4, marked (40–50% affected); 5, severe (>50% affected). * statistically significant difference with $p < 0.05$ (Wilcoxon Signed Rank Test).

	Overall score	Regeneration	Degeneration	Necrosis	Epithelial cell lose	Pigment
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MCD	3 (1-4)*	2 (2-3)*	2 (1-3)*	2 (1-3)*	3 (1-4)*	3 (1-4)*

Images were taken at 40× magnification. Higher magnification (200×) images of glomeruli changes were captured and shown as insert to the pictures (A, control kidney; B. NASH kidney).

on clearance of creatinine) and suffered from increased albuminuria and CKD (chronic kidney disease). Moreover, obesity, which is a common comorbidity, has been recently linked with rapid loss of kidney function [42]. Such data correspond with detection of overall kidney impairment observed in our studies [17]. Further research is needed to enlighten potentially causative mechanisms by which NASH could contribute to CKD pathogenesis.

In agreement with preferential renal excretion of adefovir, we have measured approximately 10 times lower concentrations of the drug in the liver of control animals in comparison to their kidneys. This difference is created by concentration of adefovir in kidney through rapid clearance by GFR and active uptake to proximal tubular cells. Uptake into liver may be limited by delayed access through portal circulation and by absence or low expression of its major uptake transporters Oat1/3. Additionally, adefovir returns from hepatocytes to the bloodstream, as there is negligible excretion into bile by apical transporting proteins, which is documented by excretion of more than 90% of the administered dose in urine.

The MCD diet further reduced liver concentrations of adefovir, and effect which may be explained by formerly described changes in hepatic drug transporting proteins. With respect to the main transporters for adefovir: Oat1/3 (uptake) and Mrp4 (efflux), the MCD diet is accompanied chiefly with up-regulation of Mrp4 protein expression and decreased mRNA expression of Oat3 [12–14]. Since Oat1 is not present in the liver, we therefore suspect that reduction in hepatic levels of adefovir may be ascribed to Mrp4 induction, which in turn effluxes adefovir from the hepatocytes back into the bloodstream. A critical role of MRP4 in liver disposition of adefovir was very recently confirmed by Liu et al. [43] in HepG2 cells. On the other hand, hepatic microcirculation and blood flow may also be compromised in NAFLD and especially in NASH [44,45]. As to whether this effect may contribute to the observed decrease in accumulation of adefovir requires further attention.

In conclusion, we found that the MCD rat model of NASH results in a decreased GFR similar to human NASH, and causes significant alternations to transporter protein expression and function in the
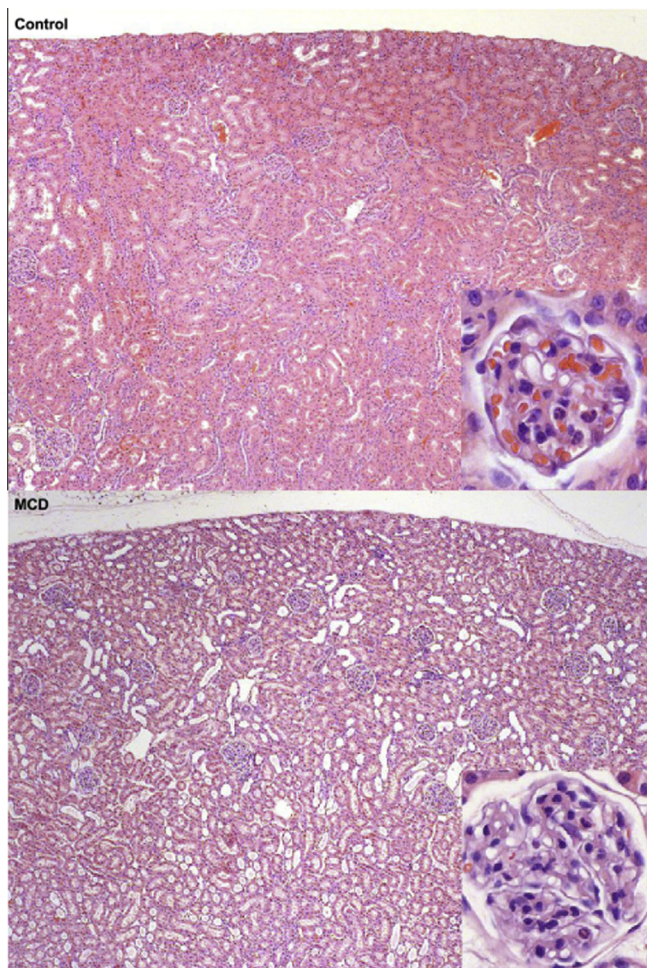
Fig. 5. Renal Morphology in control and NASH rats. Renal injury was visualized by light microcsopy and scored according to various parameters, detailed in Table 2. Images were taken at 40× magnification. Higher magnification (200×) images of glomeruli were captured and shown as insert to the pictures (A, control kidney; B. NASH kidney). Error bars are 200 μm. Definitive renal injury was visualized by light microcsopy and scored according to various parameters, detailed in Table 2.

kidney. Taken together, these findings show that NASH can significantly alter the renal elimination of xenobiotics, through both glomerular filtration and tubular secretion, without a change in urine volume. Our results point to the influence of hepatic dysfunction on extrahepatic pharmacokinetic parameters that could have a profound impact on exposure and the potential for toxicity. It will be important to investigate the relationship between NASH and the renal clearance of numerous drugs that may provide the mechanistic basis for variable drug response.

Funding

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Author's contributions

T. Laho: designed and performed the experiments and data analysis, and wrote the manuscript. J.D. Clarke: designed and performed the experiments and data analysis. A.L. Dzierlenga: performed the experiments and data analysis and wrote the manuscript. H. Li: performed the experiments and data analysis. D.M. Klein: contributed to the experiments and performed data analysis. M. Goedken: performed data acquisition. S. Micuda: performed the data analysis and provided funding support and critical revision of the manuscript. N.J. Cherrington: provided funding support and study supervision and performed a critical revision of the manuscript.

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