Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 540 (2006) 168–174
www.elsevier.com/locate/ejphar

Tubular transporters and clearance of adefovir

Aude Servais [a,1], Philippe Lechat [b,*], Noël Zahr [b], Saik Urien [b], Guy Aymard [b], Marie Chantal Jaudon [c], Gilbert Deray [a], Corinne Isnard Bagnis [a]

[a] Nephrology Department, Pitié-Salpêtrière University Hospital, 47 Boulevard de l'Hôpital, 75013, Paris, France
[b] Pharmacology Department, Pitié-Salpêtrière University Hospital, 47 Boulevard de l'Hôpital, 75013, Paris, France
[c] Biochemistry Department, Pitié-Salpêtrière University Hospital, 47 Boulevard de l'Hôpital, 75013, Paris, France

Received 8 December 2005; received in revised form 11 April 2006; accepted 28 April 2006
Available online 6 May 2006

Abstract

Adefovir is transported by the organic anion transporter (OAT1) and the multidrug resistant protein (MRP2, 4 and 5). We studied adefovir clearance in rat after inhibition of transporters by probenecid and in mutant transport-deficient (TR−) rats, in which MRP2 is lacking. After treatment by probenecid or placebo, pharmacokinetics of adefovir 10 mg/kg was studied via population nonlinear mixed effect modeling. The fraction of drug excreted in the urine was low. Renal clearance of adefovir was significantly lower ($P<0.05$) in probenecid TR− rats (0.03±0.02 l/h) than in normal control (0.09±0.05 l/h), in normal probenecid (0.10±0.07 l/h) and in TR− control rats (0.13±0.07 l/h). In vivo in rats MRP2 mutation alone did not affect adefovir clearance suggesting that MRP2 does not play a critical role in the secretion of adefovir. Additional pharmacological inhibition of transporters decreased renal clearance, which may reflect inhibition of compensating transport mechanisms activated when MRP2 is lacking.

Keywords: Organic anion transporter; Probenecid; TR− rat; Adefovir clearance

1. Introduction

Adefovir [9-[2-(phosphonomethoxy)ethyl]adenine], an acyclic phosphonate analogue, is the newest approach to the treatment of hepatitis B virus infection, especially in lamivudine-resistant mutant and in Human Immunodeficiency Virus-1 (HIV-1) co-infected patients (Benhamou et al., 2001; Squires, 2001). Drugs belonging to this family are currently becoming the cornerstone of antiretroviral therapy. But, they have shown potential proximal tubular toxicity (Hannon et al., 2004; Izzedine et al., 2004; Kahn et al., 1999). In order to take advantage of their interesting antiviral properties, it is important to limit their potential renal side effects, these goals being aimed at by achieving optimal drug dosage.

Adefovir is transported in vitro by tubular transporters, namely the organic anion transporter (OAT1) (Cihlar et al., 1999b) and the multidrug resistant protein (MRP2, 4 and 5) (Miller, 2001; Reid et al., 2003; Wijnholds et al., 2000). The assessment of interaction of adefovir with P glycoprotein and MRP1 yielded negative results (Dallas et al., 2004). It has been suggested that adefovir may inhibit transport of MRP2 substrates in killifish tubules (Miller, 2001). In this model, adefovir enters the cells on OAT1 and exits on MRP2. The ATP-driven drug efflux pump MRP4 and potentially MRP2 are transporters responsible for the efflux from cells of nucleoside monophosphates and their analogs. Because they mediate the efflux of adefovir from cells, competitive interactions at transporter may result in reduced efflux and intracellular accumulation (Schuetz et al., 1999). The human organic anion transporter 1 (hOAT 1), localized in the basolateral membrane of renal tubular cells (Tojo et al., 1999; Uwai et al., 1998), is responsible for entry into the tubular cells. The potential for adefovir toxicity is increased in various types of mammalian cells constitutively expressing hOAT 1. Renal toxicity was related to considerable accumulation in the renal tubules associated with active transport into the tubular cell by the organic anion transport

Abbreviations: TR− rat, mutant transport-deficient rat.

* Corresponding author. Tel.: +33 1 42 16 16 82; fax: +33 1 42 16 16 88.
E-mail address: philippe.lechat@psl.ap-hop-paris.fr (P. Lechat).
[1] Person to receive reprint requests.

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.04.047

system OAT1 (Cihlar et al., 1999; Ho et al., 2000). It has been demonstrated that hOAT1 specific inhibitors may reduce adefovir induced cytotoxicity (Cihlar et al., 2001). Recently, probenecid has emerged as a potential nephroprotectant when prescribed together with the antiviral drug cidofovir. Patients treated with cidofovir may develop nephrotoxicity, as a result of extensive accumulation in tubular cells associated with the process of active secretion. Probenecid is now co-administered with cidofovir to inhibit tubular organic anion transport, thus reducing its potential for nephrotoxicity (Cundy et al., 1996; Lacy et al., 1998; Lalezari et al., 1995). It appears that understanding how concomitant tubular-secreted drugs combination influences their renal and metabolic clearances is of the utmost importance in terms of efficacy and toxicity.

In the past few years, considerable progress has been made in the cloning of these transporters and their functional characterization (Burckhardt and Wolff, 2000; Inui et al., 2000; Russel et al., 2002). A major challenge is now to understand how these transporters work in vivo in order to optimize the clinical use of anionic substrates. Many clinically significant transporter-mediated drug interactions have been described (Mulato and Cihlar, 2000). It has been shown that drugs regularly used concomitantly in hepatitis B virus and HIV-infected patients may compete on different transporters. The clinical consequences of these interactions in vivo are not very well documented. Prediction of the in vivo kinetic profile of drugs in animal models is an important step towards establishing appropriate strategy for individual patient therapy.

The purpose of the present work was to study the impact of transporters OAT1 and MRP inhibition by probenecid on adefovir clearance. We studied adefovir pharmacokinetics in normal Wistar rats (Shaw et al., 1997) and in mutant transport-deficient rats (TR−) (Masereeuw et al., 2003) treated with probenecid. We also describe the essential features of mutant rats compared to normal rats treated by probenecid in order to better analyze the mechanism of action of that drug which is not well established.

2. Methods

2.1. Animals and experimental groups

We randomized for this study 40 adult male Wistar rats (329±6 g) provided by Charles River laboratories (L'Arbresle, France) and 32 mutant transport-deficient rats, TR− (333±5 g), obtained from Harlan (Gannat, France) into 4 experimental groups: normal control rats (N=22), normal probenecid rats (N=18), TR− control rats (N=22) and TR− probenecid rats (N=10). The animals were housed under controlled environmental conditions (22±1 °C ambient temperature, 60% relative humidity, 12:12 h light–dark cycle, food and water ad libitum) for the whole study. All the procedures involving animals are in compliance with the institutional guidelines and with national and international laws and policies.

2.2. Drugs administration and sample collection

After a 4 day treatment by 100 mg/kg of probenecid administrated every morning by oral gavage or placebo, adefovir (10 or 30 mg/kg) was administrated via tail artery to Wistar male rats (N=37) or TR− rats (N=17) 60 min after probenecid gavage. Animals were placed into metabolic cages. Sequential blood samples were collected over 48 h (5 or 6 per rat) and urine samples were collected over the same period. After short anesthesia with isoflurane (Forene®), blood samples were collected into heparinized tubes from each group of animals by retro-orbital sinus puncture. Blood (0.2 ml) was processed immediately for plasma by centrifugation at 2700×g for 10 min. Plasma samples were frozen and maintained at −80 °C until analyzed. Plasma concentrations of probenecid were measured in 10 normal adefovir treated rats (17 plasma samples) and in 10 TR− (18 plasma samples) adefovir treated rats over 24 h after probenecid gavage.

2.3. Determination of adefovir dosage in plasma and urine samples

Adefovir was assayed from plasma and urine samples by a previously described high performance liquid chromatography (HPLC) method (Jullien et al., 2003) with modifications.

The HPLC system consisted of a 1525 binary pump, a Wisp 717 Plus autosampler injector set, a 2475 Multi λ fluorescence detector set at 236 and 420 nm for excitation and emission wavelengths respectively and a Millenium software for the acquisition and the treatment of chromatograms. Chromatographic separation was achieved on a reversed phase C_8 plus Satisfaction® column (250×3 mm, 5 µm; Cluzeau, Sainte Foy la Grande France). The mobile consisted of sodium acetate buffer (50 mM) containing 5 mM of tetrabutylammonium phosphate adjusted at pH 5.5. The flow rate was 0.5 ml/min. In hemolysis tubes, 100 µl of a working solution of tenofovir (used as an internal standard) were added to 50 µl of plasma or urine samples and to 600 µl of methanol. Samples were then vortex-mixed 10 s and centrifuged for 10 min. The supernatant was evaporated to dryness. The residues were redissolved in 260 µl of sodium acetate buffer. Samples were then purified, vortex-mixed and centrifuged. A 150 µl volume of the supernatant was transferred to a screw-cap glass tube containing 50 µl of the derivatization reagent. After adding the screw-cap, the tubes were incubated at 80 °C for 50 min. Derivatized samples were then injected in the chromatographic system. Retention time of adefovir was 6.1 min.

2.4. Population pharmacokinetic modeling

Data was analyzed with a population based approach using the nonlinear mixed effect modeling software program NONMEM (version V, level 1.1, double precision) (Beal and Sheiner, 1998). The first-order conditional estimation (FOCE) with the INTERACTION option was used. Diagnostic graphics and distribution statistics were obtained using the R program (Ihaka and Gentleman, 1996). Plasma adefovir concentrations versus

time from intra-arterial administrations were fitted using the nonlinear mixed effect modeling software program NONMEM subroutine ADVAN4 TRANS4. Parameters of the model were V_1 and V_2, central and peripheral distribution volumes, CL and Q, elimination and intercompartmental clearances. Several error models were investigated (i.e., proportional, exponential and additive error models) to describe inter-subject (ISV) and residual variability.

Effect of categorical covariates–normal or TR− rats, receiving or not probenecid, adefovir dose of 10 or 30 mg/kg– was investigated by estimating distinct parameter value for each modality.

Covariates were selected in the final population model if (i) their effect was biologically plausible, (ii) they produced a minimum reduction of 4 units in the objective function value (OFV) and (iii) they produced a reduction in the variability of the pharmacokinetic parameters, assessed by the associated inter-subject variability. For evaluation of the goodness-of-fit, the following graphs were considered: observed concentrations versus predictions (PRED–OBS), weighted residuals (WRES) versus time and weighted residuals versus PRED (WRES–PRED) as well as the corresponding graphs issued from the POSTHOC estimation step (Bayesian estimation).

2.5. Drugs

Commercially available probenecid was purchased from Sigma Company (Saint Quentin Falavier, France). Probenecid powder (300 mg) was diluted in 1 ml of 1 M NaOH solution, further diluted with physiological saline and then adjusted to a physiological pH with HCl solution before oral gavage administration. Adefovir was provided by Gilead Sciences Inc. (Foster, LA, United States). It was diluted in NaOH, diluted with physiological saline and then adjusted to pH 7.4 before tail artery administration. Isoflurane (Forene®) was provided by Abbott (Rungis, France).

2.6. MRP2 inhibition evaluation

TR− rats in which MRP2 is lacking are known to have a hyperbilirubinemia and an increased urinary coproporphyrin isomer I excretion, a pattern similar to Dubin-Johnson syndrome in humans. In order to analyze the molecular mechanism of action of probenecid, we studied the rate of excretion of urinary coproporphyrins (Frank et al., 1990; Jansen et al., 1985) and serum bilirubin in mutant-deficient TR− rats compared to normal rats treated by probenecid during 4 days. For coproporphyrins dosage, urine was collected in the dark from rats kept in metabolic cages. Urinary coproporphyrins were detected by HPLC. The fluorescence detector was set at 365 and 624 nm for excitation and emission wavelengths respectively. Chromatographic separation was achieved on a reversed phase dC_{18} Atlantis® column (250 × 4.6 mm, 5 µm). The mobile phase was a mixture of acetonitrile (A) and sodium acetate buffer (B). A linear gradient elution of 1.2 ml/min consisting of 30/70 to 47/53 (A/B) was used during the first 10 min; followed by a linear gradient elution of 1.3 to 1.5 ml/min consisting of 70/30 (A/B) for the next 5 min. Then, the system was reequilibrated for an additional 6 min with the initial conditions. In hemolysis tubes, 1 ml of methanol was added to 250 µl of acidified urine sample. Samples were vortex-mixed for 10 s and evaporated to dryness. The residues were redissolved in 1 N HCl. Samples were then purified by addition of chloroform, vortex-mixed for 10 s and centrifuged. A volume of 50 µl of the supernatant was injected in the chromatographic system. Retention times of coproporphyrins I and III were 8.0 and 9.1 min, respectively. For bilirubin dosage, blood was drawn from the supra-orbital plexus during short anesthesia. Serum bilirubin was measured by colorimetry on a Modular Analyzer (Roche® Diagnostics).

3. Results

For the pharmacokinetic analysis, 299 plasma samples from 72 rats were available. Adefovir concentration–time profiles were best described by a two-compartment model, which served as the base model for further covariate search and model refinement. ISVs could not be estimated for Q and V_2, and deletion of these parameters did not modify the OFV value. The mean population pharmacokinetic parameters of adefovir are shown in Table 1. No effect of adefovir dose was observed on pharmacokinetic parameters in covariate analysis, indicating that the pharmacokinetics was linear. The combination of the normal/TR− and probenecid/no probenecid features provided four subgroups. The only covariate that influenced adefovir pharmacokinetics was probenecid, producing a total clearance decrease in TR− rats and a total clearance increase in normal rats.

Individual drug exposures in plasma were derived from Bayesian (POSTHOC) estimations of pharmacokinetic parameters. Typical adefovir concentration–time courses in each group are shown in Fig. 1. Table 2 summarizes the total, renal and non-renal clearances and the fraction of drug excreted in urine. Total clearance in TR− control rats was not different from normal control rats. Total clearance in probenecid TR− rats was

Table 1
Mean population pharmacokinetic parameters of adefovir after injection of 10 mg/kg or 30 mg/kg in controls, probenecid and TR− rats

Parameter	Estimate	S.E.M
Pharmacokinetic		
CL tot (l/h)	0.36	0.03
V_1 (l)	0.45	0.04
Q (l/h)	0.20	0.03
V_2 (l)	4.58	1.04
Inter-subject variability		
Ω^2 (CL tot)	0.03	0.009
Ω^2 (V1)	0.18	0.05
Intra-subject variability		
σ^2 (proportional)	0.18	0.02
σ^2 (additive, fixed)	0.0001	

S.E.M, standard error of measurement; V_1, central volume of distribution; V_2, peripheral volume of distribution; Q, intercompartmental clearance; CL tot, total clearance; Ω^2, inter-subject variability; σ^2, residual error.

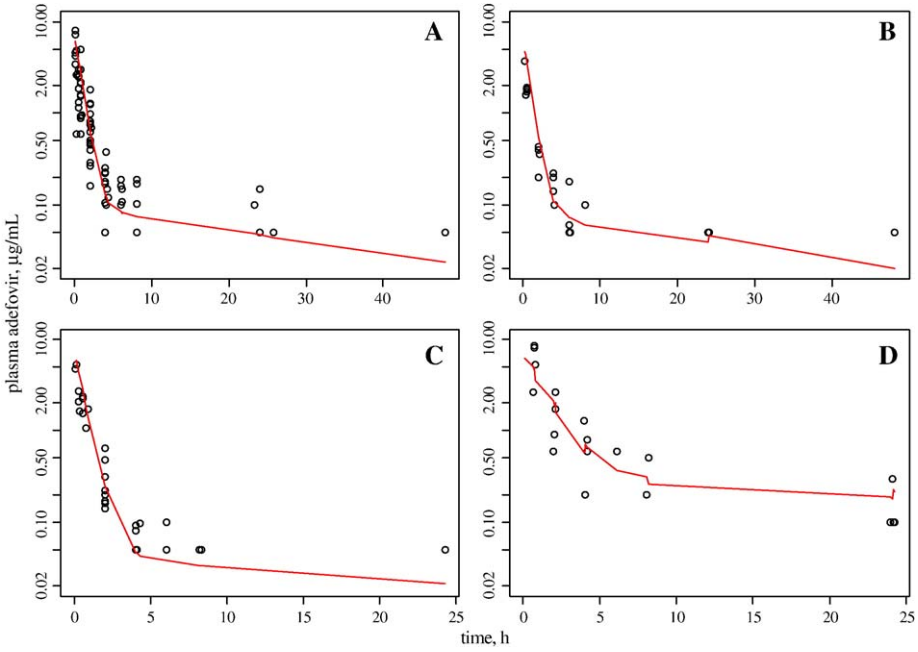

Fig. 1. Plasma concentration of adefovir after injection of 10 mg/kg of adefovir via tail artery in control or TR− rats with or without pre-treatment by probenecid. The solid line represents the population-predicted concentrations. (Panel A) Normal control rats; (B) normal probenecid rats; (C) TR− control rats; (D) TR−+probenecid rats.

significantly decreased ($P<0.001$) versus normal control rats, versus normal probenecid and versus TR− control rats. Total clearance of adefovir was significantly higher ($P<0.001$) in normal probenecid rats compared to normal controls.

Renal clearance of adefovir in TR− control rats was not different from normal control rats. It was significantly lower ($P<0.05$) in probenecid TR− rats than in normal control rats, in normal probenecid rats and in TR− control rats. The fraction of drug excreted in urine was low (Table 2).

In probenecid TR− rats, non-renal clearance was significantly decreased compared to normal and TR− controls and to normal probenecid. It increased significantly in normal probenecid rats compared to controls and to TR− rats ($P<0.001$).

Mean plasma concentration of probenecid in normal adefovir treated rats was 19.6 ng/ml and 14.8 ng/ml in TR− adefovir treated rats. Thus, they were in a range of effective concentrations [10 to 50 ng/ml] after oral gavage with probenecid.

Serum bilirubin levels were greatly elevated in TR− rats compared to normal rats. It was not increased in normal rats after a 4-day treatment by probenecid (Table 3).

Urinary coproporphyrin excretion was measured and the results are depicted in Fig. 2. Total urinary coproporphyrin excretion was similar in TR− rats and in normal rats with or without treatment by probenecid, but total and relative urinary coproporphyrin isomer I excretion was increased in mutant rats. It was not modified in normal rats or TR− rats after treatment by probenecid.

4. Discussion

The purpose of this work was to develop an animal model to study in vivo the interactions between adefovir and organic anion transporters. We showed that MRP2 mutation alone did not affect adefovir clearance. After an additional pharmacological inhibition of organic anion transporters in MRP2 deficient rats renal clearance was decreased. In contrast probenecid alone in normal rats increased the non-renal clearance.

Table 2
Pharmacokinetic parameters after injection of adefovir via tail artery in control or TR− rats pre-treated by probenecid or by placebo

	Total CL (l/h)	EF u (%)	"Non-renal" CL (l/h)	Renal CL (l/h)
Normal control ($N=22$)	0.38±0.10	26±13	0.29±0.11	0.09±0.05
Normal+probenecid ($N=18$)	0.58±0.09[a]	18±12	0.47±0.10[a]	0.10±0.07
TR− control ($N=22$)	0.38±0.09	34±18	0.25±0.10	0.13±0.07
TR−+probenecid ($N=10$)	0.19±0.08[a,b,c]	17±10	0.16±0.06[a,b]	0,03±0.02[a,c]

[a] $P<0.001$ versus normal control rats; [b] $P<0.001$ versus normal+probenecid rats; [c] $P<0.001$ versus TR− control rats. CL, clearance; EF u, fraction of drug excreted in urine. Mean±S.E.M.

Table 3
Serum bilirubin (in µmol/l) in TR− rats and in normal rats before (D0) and after a 4-day (D4) treatment by probenecid

	TR−	Normal control D0	Normal probenecid D0	Normal control D4	Normal probenecid D4
Bilirubinemia (µmol/l)	86.2±11.7	1.0±0.6	0.1±0.0	1.0±0.7	1.3±0.8

Mean±S.E.M.

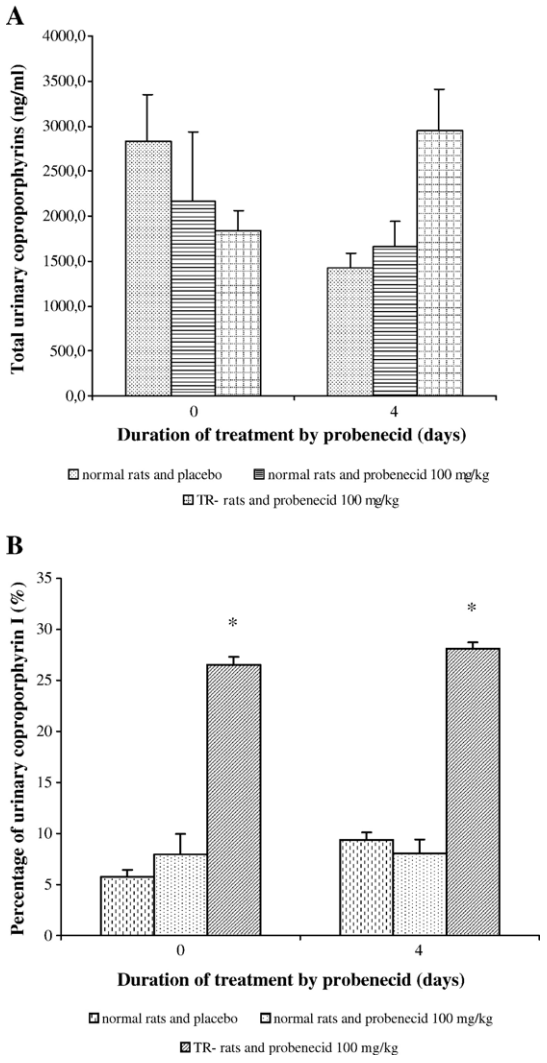

Fig. 2. (Panel A) Urinary total coproporphyrin in TR− rats and in normal rats before and after a 4-day treatment by 100 mg/kg of probenecid. (Panel B) Urinary coproporphyrin I compared to coproporphyrin II in TR− rats and in normal rats before and after a 4-day treatment by 100 mg/kg of probenecid. Urine was collected in the dark. Coproporphyrin isomers were determined by high-performance liquid chromatography. Means ± S.E.M. *$P<0.01$ compared to normal rats treated by probenecid or by placebo.

In vitro studies indicate that active tubular secretion is involved in the elimination of adefovir via OAT1, OAT3, MRP4, MRP5 and MRP2 (in the killifish tubule model). But in vivo in rats the lack of MRP2 does not affect renal clearance of adefovir according to our experiments. Therefore MRP2 does not play an important role in adefovir tubular secretion. However, the absence of a transporter in a knockout model does not rule out the significance of this transporter in physiological settings of xenobiotic renal excretion. Indeed, MRP2 deficiency has no effect on *p*-aminohippuric acid (PAH) excretion but bile duct ligation which significantly upregulates MRP2 levels in the proximal tubule markedly increases PAH excretion (Tanaka et al., 2002). MRP2 may actually play a role in normal rats and the lack of difference in renal clearance of adefovir in TR− rats might be due to the presence of an efficient compensatory renal efflux mechanism. Additional transporters compensating for the loss of MRP2 may be involved. Kidney perfusion studies and transport studies with isolated proximal tubules have indicated that the absence of MRP2 from TR− kidneys does not affect renal excretion of some organic anions (Van Aubel et al., 2000). It has been shown that MRP4 could be involved in this compensatory mechanism for adefovir renal excretion (Masereeuw et al., 2003; Rius et al., 2003; Smeets et al., 2004). Additional transporters inhibition (including MRP4) by probenecid may explain that renal clearance is decreased in probenecid TR− rats. The absence of similar mechanisms of compensation and upregulation of other transporters in normal rats may explain the absence of effect of probenecid on adefovir renal clearance. In contrast, it has been demonstrated in several studies (Hosoyamada et al., 1999) that rat OAT1 is sensitive to probenecid in vitro and that probenecid inhibits the in vitro transport of adefovir by rat OAT1 (Cihlar et al., 1999; Jutabha et al., 2003). Mean plasma concentration of probenecid was in a range of effective concentrations in our study. These experimental results confirm that in vivo in rats OAT1 is not the only transporter that participate in tubular secretion of adefovir.

The same effect is observed on the non-renal clearance of adefovir which is similar between normal controls and TR− rats but which is decreased in TR− rats treated by probenecid. This non-renal clearance could be a “metabolic” clearance in hepatocytes or in lymphocytes. The lack of a transporter could affect expression and possibly also distribution between apical and basolateral membranes of other transporters which could explain the differences of action of probenecid observed between normal and TR− rats. This appears to indicate a complex phenotype of TR− rats, possibly due to the effect of MRP2 deficiency on the level of expression of other transporters, likely in multiple compartments.

In our study, the fraction of drug excreted in urine is low and similar both in TR−, normal probenecid and normal control rats. In humans, the majority of an adefovir dose is excreted in urine within 24 h of intravenous administration: 98% of the intravenous dose are recovered unchanged in the urine by 24 h postdosing (Cundy et al., 1995). Species dependence in transporter expression, affinity and distribution between liver and kidney may explain this difference (Russel et al., 2002).

The molecular mechanisms of action of probenecid has never been really explained and its inhibitory effect on transporters is aspecific (Masereeuw et al., 2000). We have compared the rate of excretion of urinary coproporphyrins and the serum bilirubin in mutant-deficient TR− rats in which MRP2 is lacking to normal rats treated by probenecid during 4 days. TR− rats exhibit a hyperbilirubinemia and an increased urinary coproporphyrin isomer I excretion, a pattern similar to Dubin-Johnson syndrome in humans (Jansen et al., 1985). As for adefovir clearance, we showed that treatment by probenecid does not have the same consequences on these biological features than the loss of MRP2. In vivo, this drug may not exhibit marked inhibitory effect on MRP2.

Non-renal clearance of 10 mg/kg adefovir is significantly increased in probenecid normal rats compared to controls. Adefovir is a prodrug, since it is an inactive compound that requires metabolic activation by cellular enzymes. The active

form is the corresponding diphosphate formed within cells by host enzymes following 2 phosphorylation steps (Merta et al., 1992). Intracellular concentrations of these phosphorylated metabolites are not measurable, and therefore no direct data are currently available on clinical exposure to them. In humans, it is unlikely that metabolism is a significant pathway of elimination (Qaqish et al., 2003). However pharmacokinetic parameters in our study suggest intracellular accumulation (in lymphocytes or in hepatocytes) and/or metabolism increase in normal rats treated by probenecid. Metabolism could be induced by the increase of intracellular concentration of adefovir. This is probably not due to MRP2 inhibition since TR− rats in which MRP2 is lacking behave similarly to controls. MRP4, MRP5 and OAT-k1 (which is specific to rats) could be involved in that difference in terms of adefovir clearance between probenecid and control rats since they play a role in adefovir extrusion from cells and can be inhibited by probenecid. The reason why such effect is not observed in TR− rats remains unknown: the elevated bilirubin level could affect metabolism.

Intracellular activation of a fraction of the dose of adefovir by cellular kinases leads to prolonged antiviral effects that are not easily predicted from conventional pharmacokinetic studies (Cundy, 1999). The observed rate of elimination from serum does not reflect the true duration of action of the drug, since antiviral effect is dependent on active phosphorylated metabolites concentration within the cell. Drug interactions on transporters could then affect efficiency as well as toxicity of adefovir.

Our study stresses the role of potential drug induced tubular interactions on therapeutic effects of nucleoside analogs. Because patients are being exposed to lifelong drug combination therapy, it is critical to document precisely tubular mechanism involved in drug's handling.

Acknowledgement

This study was supported by Gilead Sciences Inc. and by the French Society of Nephrology. This work has been presented as an abstract at the French Society of Nephrology annual meeting, Marseille, France, September 2004 and at the 3rd World Congress of Nephrology, Singapore, June 2005.

References

Beal, S., Sheiner, L., 1998. NONMEM User's Guide. University of California, San Francisco, CA, USA.

Benhamou, Y., Bochet, M., Thibault, V., Calvez, V., Fievet, M.H., Vig, P., Gibbs, C.S., Brosgart, C., Fry, J., Namini, H., Katlama, C., Poynard, T., 2001. Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. Lancet 358, 718–723.

Burckhardt, G., Wolff, N.A., 2000. Structure of renal organic anion and cation transporters. Am. J. Physiol., Renal Physiol. 278, F853–F866.

Cihlar, T., Lin, D.C., Pritchard, J.B., Fuller, M.D., Mendel, D.B., Sweet, D.H., 1999. The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. Mol. Pharmacol. 56, 570–580.

Cihlar, T., Ho, E.S., Lin, D.C., Mulato, A.S., 2001. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleosides Nucleotides Nucleic Acids 20, 641–648.

Cundy, K.C., 1999. Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir. Clin. Pharmacokinet. 36, 127–143.

Cundy, K.C., Barditch-Crovo, P., Walker, R.E., Collier, A.C., Ebeling, D., Toole, J., Jaffe, H.S., 1995. Clinical pharmacokinetics of adefovir in human immunodeficiency virus type 1-infected patients. Antimicrob. Agents Chemother. 39, 2401–2405.

Cundy, K.C., Li, Z.H., Lee, W.A., 1996. Effect of probenecid on the distribution, metabolism, and excretion of cidofovir in rabbits. Drug Metab. Dispos. 24, 315–321.

Dallas, S., Schlichter, L., Bendayan, R., 2004. Multidrug resistance protein (MRP) 4- and MRP 5-mediated efflux of 9-(2-phosphonylmethoxyethyl) adenine by microglia. J. Pharmacol. Exp. Ther. 309, 1221–1229.

Frank, M., Doss, M., de Carvalho, D.G., 1990. Diagnostic and pathogenetic implications of urinary coproporphyrin excretion in the Dubin-Johnson syndrome. Hepatogastroenterology 37, 147–151.

Hannon, H., Bagnis, C.I., Benhamou, Y., Beaufils, H., Sullivan, M., Brosgart, C., Izzedine, H., Poynard, T., Deray, G., 2004. The renal tolerance of low-dose adefovir dipivoxil by lamivudine-resistant individuals co-infected with hepatitis B and HIV. Nephrol. Dial. Transplant. 19, 386–390.

Ho, E.S., Lin, D.C., Mendel, D.B., Cihlar, T., 2000. Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. J. Am. Soc. Nephrol. 11, 383–393.

Hosoyamada, M., Sekine, T., Kanai, Y., Endou, H., 1999. Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. Am. J. Physiol. 276, F122–F128.

Ihaka, R., Gentleman, R., 1996. R: a language for data analysis and graphics. J. Comput. Graph. Stat. 5, 299.

Inui, K.I., Masuda, S., Saito, H., 2000. Cellular and molecular aspects of drug transport in the kidney. Kidney Int. 58, 944–958.

Izzedine, H., Hulot, J.S., Launay-Vacher, V., Marcellini, P., Hadziyannis, S.J., Currie, G., Brosgart, C.L., Westland, C., Arterbrun, S., Deray, G., 2004. Renal safety of adefovir dipivoxil in patients with chronic hepatitis B: two double-blind, randomized, placebo-controlled studies. Kidney Int. 66, 1153–1158.

Jansen, P.L., Peters, W.H., Lamers, W.H., 1985. Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport. Hepatology 5, 573–579.

Jullien, V., Treluyer, J.M., Pons, G., Rey, E., 2003. Determination of tenofovir in human plasma by high-performance liquid chromatography with spectrofluorimetric detection. J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 785, 377–381.

Jutabha, P., Kanai, Y., Hosoyamada, M., Chairoungdua, A., Kim do, K., Iribe, Y., Babu, E., Kim, J.Y., Anzai, N., Chatsudthipong, V., Endou, H., 2003. Identification of a novel voltage-driven organic anion transporter present at apical membrane of renal proximal tubule. J. Biol. Chem. 278, 27930–27938.

Kahn, J., Lagakos, S., Wulfsohn, M., Cherng, D., Miller, M., Cherrington, J., Hardy, D., Beall, G., Cooper, R., Murphy, R., Basgoz, N., Ng, E., Deeks, S., Winslow, D., Toole, J.J., Coakley, D., 1999. Efficacy and safety of adefovir dipivoxil with antiretroviral therapy: a randomized controlled trial. Jama 282, 2305–2312.

Lacy, S., Hitchcock, M., Lee, W., Tellier, P., Cundy, K., 1998. Effect of oral probenecid coadministration on the chronic toxicity and pharmacokinetics of intravenous cidofovir in cynomolgus monkeys. Toxicol. Sci. 44, 97–106.

Lalezari, J.P., Drew, W.L., Glutzer, E., James, C., Miner, D., Flaherty, J., Fisher, P.E., Cundy, K., Hannigan, J., Martin, J.C., et al., 1995. (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (cidofovir): results of a phase I/II study of a novel antiviral nucleotide analogue. J. Infect. Dis. 171, 788–796.

Masereeuw, R., van Pelt, A.P., van Os, S.H., Willems, P.H., Smits, P., Russel, F.G., 2000. Probenecid interferes with renal oxidative metabolism: a potential pitfall in its use as an inhibitor of drug transport. Br. J. Pharmacol. 131, 57–62.

Masereeuw, R., Notenboom, S., Smeets, P.H., Wouterse, A.C., Russel, F.G., 2003. Impaired renal secretion of substrates for the multidrug resistance protein 2 in mutant transport-deficient (TR−) rats. J. Am. Soc. Nephrol. 14, 2741–2749.

Merta, A., Votruba, I., Jindrich, J., Holy, A., Cihlar, T., Rosenberg, I., Otmar, M., Herve, T.Y., 1992. Phosphorylation of 9-(2-phosphonomethoxyethyl) adenine and 9-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)adenine by AMP(dAMP) kinase from L1210 cells. Biochem. Pharmacol. 44, 2067–2077.

Miller, D., 2001. Nucleoside phosphonate interactions with multiple organic anion transporters in renal proximal tubule. J. Pharmacol. Exp. Ther. 299, 567–574.

Mulato, A., Cihlar, H., 2000. Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. J. Pharmacol. Exp. Ther. 295, 10–15.

Qaqish, R.B., Mattes, K.A., Ritchie, D.J., 2003. Adefovir dipivoxil: a new antiviral agent for the treatment of hepatitis B virus infection. Clin. Ther. 25, 3084–3099.

Reid, G., Wielinga, P., Zelcer, N., De Haas, M., Van Deemter, L., Wijnholds, J., Balzarini, J., Borst, P., 2003. Characterization of the transport of nucleoside analog drugs by the human multidrug resistance proteins MRP4 and MRP5. Mol. Pharmacol. 63, 1094–1103.

Rius, M., Nies, A.T., Hummel-Eisenbeiss, J., Jedlitschky, G., Keppler, D., 2003. Cotransport of reduced glutathione with bile salts by MRP4 (ABCC4) localized to the basolateral hepatocyte membrane. Hepatology 38, 374–384.

Russel, F., Masereeuw, R., Van Aubel, R., 2002. Molecular aspects of renal anionic drug transport. Annu. Rev. Physiol. 64, 563–594.

Schuetz, J.D., Connelly, M.C., Sun, D., Paibir, S.G., Flynn, P.M., Srinivas, R.V., Kumar, A., Fridland, A., 1999. MRP4: a previously unidentified factor in resistance to nucleoside-based antiviral drugs. Nat. Med. 5, 1048–1051.

Shaw, J.P., Louie, M.S., Krishnamurthy, V.V., Arimilli, M.N., Jones, R.J., Bidgood, A.M., Lee, W.A., Cundy, K.C., 1997. Pharmacokinetics and metabolism of selected prodrugs of PMEA in rats. Drug Metab. Dispos. 25, 362–366.

Smeets, P.H., van Aubel, R.A., Wouterse, A.C., van den Heuvel, J.J., Russel, F. G., 2004. Contribution of multidrug resistance protein 2 (MRP2/ABCC2) to the renal excretion of *p*-aminohippurate (PAH) and identification of MRP4 (ABCC4) as a novel PAH transporter. J. Am. Soc. Nephrol. 15, 2828–2835.

Squires, K.E., 2001. An introduction to nucleoside and nucleotide analogues. Antivir. Ther. 6 (Suppl 3), 1–14.

Tanaka, Y., Kobayashi, Y., Gabazza, E.C., Higuchi, K., Kamisako, T., Kuroda, M., Takeuchi, K., Iwasa, M., Kaito, M., Adachi, Y., 2002. Increased renal expression of bilirubin glucuronide transporters in a rat model of obstructive jaundice. Am. J. Physiol.: Gasterointest. Liver Physiol. 282, G656–G662.

Tojo, A., Sekine, T., Nakajima, N., Hosoyamada, M., Kanai, Y., Kimura, K., Endou, H., 1999. Immunohistochemical localization of multispecific renal organic anion transporter 1 in rat kidney. J. Am. Soc. Nephrol. 10, 464–471.

Uwai, Y., Okuda, M., Takami, K., Hashimoto, Y., Inui, K., 1998. Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. FEBS Lett. 438, 321–324.

Van Aubel, R.A., Masereeuw, R., Russel, F.G., 2000. Molecular pharmacology of renal organic anion transporters. Am. J. Physiol,. Renal Physiol. 279, F216–F232.

Mol, C., van Deemter, L., de Haas, M., Scheffer, G., Baas, F., Beijnen, J., Scheper, R., Hatse, S., De Clercq, E., Balzarini, J., Borst, P., 2000. Multidrug-resistance protein 5 is a multispecific organic anion transporter able to transport nucleotide analogs. Proc. Natl. Acad. Sci. U. S. A. 97, 7476–7481.