Effects of 1α,25-Dihydroxyvitamin D₃ on Intestinal Absorption and Disposition of Adefovir Dipivoxil and Its Metabolite, Adefovir, in Rats

In Soo Yoon, a Jun-Hyeng Son, b Sang-Bum Kim, b Min-Koo Choi, c,d, and Han-Joo Maeng a,c,d

a College of Pharmacy and Natural Medicine Research Institute, Mokpo National University; Muan-gun, Jeonnam 534–729, Republic of Korea; b College of Pharmacy, Seoul National University; Seoul 151–742, Republic of Korea; c College of Pharmacy, Dankook University; Cheonan 330–714, Republic of Korea; and d College of Pharmacy, Gachon University; 191 Hambakmoei-ro, Yeonsu-gu, Incheon 406–799, Republic of Korea.

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The aim of this study was to investigate the effect of 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), an active form of vitamin D, on the oral absorption and disposition of adefovir dipivoxil (P-glycoprotein (P-gp) substrate) and its major active metabolite, adefovir (multidrug resistance-associated protein 4 (Mrp4) substrate), in rats. The pharmacokinetics of intravenous adefovir and oral adefovir dipivoxil was evaluated in control and 1,25(OH)₂D₃-treated rats. The intestinal absorption of adefovir dipivoxil was investigated through an in situ closed loop study, and the tissue distribution of adefovir after oral administration of adefovir dipivoxil was evaluated in the two groups. There was no significant difference in pharmacokinetic parameters of intravenous adefovir between the two groups. Importantly, the total area under the plasma concentration–time curve from time zero to time infinity (AUC), peak plasma concentration (Cₘₚₙₜ), and extent of absolute oral bioavailability (F) of adefovir after oral administration of adefovir dipivoxil were significantly higher in 1,25(OH)₂D₃-treated rats than in control rats. In the in situ closed loop study, there was no significant difference in the remaining fraction of adefovir dipivoxil in the duodenum, jejunum and ileum loops between the two groups. In the tissue distribution study after oral administration of adefovir dipivoxil, the tissue-to-plasma partition coefficients of adefovir in the liver, brain, kidney, and intestine were significantly lower in the 1,25(OH)₂D₃-treated rats than in control rats. The present study indicates that 1,25(OH)₂D₃ treatment can enhance the oral absorption of adefovir dipivoxil, likely via the induction of basolateral Mrp4 function in rat intestine. However, the impact of 1,25(OH)₂D₃ treatment on the pharmacokinetics of intravenous adefovir was limited. These results could lead to further studies in clinically significant P-gp and/or Mrp4-mediated 1,25(OH)₂D₃–drug interactions.

Key words adefovir dipivoxil; adefovir; 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃); P-glycoprotein; multidrug resistance-associated protein 4; intestine

Vitamin D is widely used as a nutraceutical in the prolongation of longevity and in the treatment of cancer.1–3) The activation of vitamin D requires consecutive metabolism to form 25-hydroxyvitamin D₃ in the liver and then 1α,25-dihydroxyvitamin D₃ (also called 1,25(OH)₂D₃ or calcitriol), the ligand of vitamin D receptor (VDR), in the kidney.4) During the past decades, significant physiological roles of VDR in calcium and bone homeostasis have been well established.5) However, it is increasingly recognized that VDR is also crucial in the regulation of drug transporters, and 1,25(OH)₂D₃-induced VDR activation.6–10) Since VDR is abundantly expressed in the intestine,11) the effects of 1,25(OH)₂D₃ (i.e., VDR activation) on the expression of intestinal drug transporters are frequently investigated. 12) 1,25(OH)₂D₃ increases the mRNA expression and function of multidrug resistance protein 1 (MDR1) in the human colorectal adenocarcinoma cell lines, LS174T and Caco-2 cells in vitro.8,11) It also increases the protein expressions of multidrug resistance-associated protein 2 and 4 (MRP2 and MRP4) in Caco-2 cells.8,13) In rats, 1,25(OH)₂D₃ treatment in vivo increases the protein expressions of Mrp2, Mrp3, Mrp4, and the oligopeptide transporter 1 (PepT1) without altering the mRNA and protein expressions of Mrp2 (P-glycoprotein; P-gp).14) Moreover, our previous studies using rat everted intestinal sac technique confirmed that the in vitro functions of rat intestinal Mrp2, Mrp4 and PepT1, but not P-gp are induced by 1,25(OH)₂D₃ treatment via VDR activation.15)

However, changes in the expression and/or in vitro functions of drug transporters do not always correlate with the fates of therapeutic agents in the whole body system. Therefore, further studies are needed on the effects of 1,25(OH)₂D₃ on transporter substrate drug pharmacokinetics in animals or humans. In particular, we focused on adefovir dipivoxil and its major active metabolite, adefovir, in this study. Adefovir, an acyclic nucleoside phosphonate analogue with antiviral activity, acts as an inhibitor of reverse transcriptase,16) and is a well-recognized MRP4/Mrp4 substrate.17,18) Since the oral absorption of adefovir is quite poor due to its negative charge on phosphate groups, its prodrug, adefovir dipivoxil, is synthesized to mask the negative charge, enhancing membrane permeability and oral bioavailability.19) The prodrug, adefovir dipivoxil, is also recognized as a P-gp substrate.20,21) Oral administration of adefovir dipivoxil is known to be rapidly converted into adefovir by esterase located in enterocytes and reaches the systemic circulation as a form of adefovir.13) Herein, we report the effects of 1,25(OH)₂D₃ on the oral absorption and disposition of adefovir dipivoxil and its major active metabolite, adefovir, in Sprague-Dawley rats. The pharmacokinetics of adefovir after the intravenous administration of adefovir dipivoxil was also evaluated in control and 1,25(OH)₂D₃-treated rats. Additiona
ally, the intestinal absorption of adefovir dipivoxil was investigated through an in situ closed loop study in the two groups. Tissue distribution of adefovir after oral administration of adefovir dipivoxil was also evaluated and compared between the two groups.

MATERIALS AND METHODS

Materials Radiolabeled [3H]adefovir dipivoxil (5.5 mCi/µmol) was purchased from PerkinElmer, Inc. (Waltham, MA, U.S.A.). Radiolabeled [3H]adefovir (10 mCi/µmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA, U.S.A.). 1,25(OH)2D3, non-radioactive adefovir dipivoxil and adefovir were purchased from Sigma-Aldrich, Co. (St. Louis, MO, U.S.A.). All other reagents were obtained from Sigma-Aldrich, Co. and Thermo Fisher Scientific, Inc. (Waltham, MA, U.S.A.).

Animals Protocols for the animal studies were handled in accordance with the guidelines for the Institutional Animal Care and Use Committee of Seoul National University (date of approval 22/07/2013; approval number SNU-130722-1). Male Sprague-Dawley rats (7–9 weeks old, 230–300 g) were purchased from Orient Bio, Inc. The rats were reared in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 20–23°C with 12-h light (07:00–19:00) and dark (19:00–07:00) cycles, and a relative humidity of 50±20%. The rats were housed in ventilated rat cages (Tecniplast U.S.A., Inc.) under filtered and pathogen-free air, with food (Agribrands Purina Chow, St. Louis, MO, U.S.A.) and water available ad libitum.

1,25(OH)2D3 and Vehicle (Corn Oil) Treatment in Rats After 1,25(OH)2D3 was dissolved in anhydrous ethanol, the concentration of 1,25(OH)2D3 was measured by spectrophotometer at 265 nm (UV-1700, Shimadzu Scientific Instruments, MD, U.S.A.). Then, the 1,25(OH)2D3 solution was diluted to 2.56 nmol/mL with a vehicle (i.e., filtered corn oil) for intraperitoneal injection. Rats (n=3–4 in each group) were given intraperitoneal injection at a 1,25(OH)2D3 dose of 0 (vehicle) or 2.56 nmol/kg in 1 mL/kg corn oil, daily for 4 consecutive days as previously reported.9,10,11

Preliminary Studies for Liver and Kidney Function in 1,25(OH)2D3-Treated Rats In order to measure liver and kidney function in 1,25(OH)2D3-treated rats, on day 5, a 24-h urine sample was collected for the measurement of the level of creatinine. And, blood sample was collected for the measurements of levels of total plasma protein, plasma albumin, blood urea nitrogen, creatinine, serum glutamate oxaloacetate transaminase (sGOT), serum glutamate pyruvate transaminase (sGPT), which was analyzed by Green Cross Reference Lab (Seoul, South Korea). For the estimation of glomerular filtration rate (GFR), creatinine clearance (CLcre) values were calculated by dividing the total amount of excreted creatinine unchanged in the urine over 24 h by the plasma creatinine level.

In Vivo Pharmacokinetic Study in Rats The femoral artery and vein were cannulated with polyethylene tubing (PE-50; Clay Adams, Parsippany, NJ, U.S.A.) under light anesthetization with zoletil (20 mg/kg, intramuscular injection).8,2 Control or 1,25(OH)2D3-treated rats (n=4 in each group) were given a single intravenous dose (15 µmol/kg as adefovir) of [3H]adefovir or an oral dose (36.6 µmol/kg as adefovir) of [3H]adefovir dipivoxil (dissolved in 0.9% NaCl-injectable solution). One hundred fifty microliters blood samples were collected via the femoral artery at 0 (to serve as control), 1, 5, 15, 30, 60, 120, 180, 240, and 360 min after intravenous injection and at 0 (to serve as control), 30, 60, 90, 120, 180, 240, 360, 480, and 720 min after oral administration. A 50-µL plasma sample was obtained by centrifugation of the blood sample at 16000×g at 4°C for 10 min. The radioactivity of [3H]adefovir in the plasma sample was measured by liquid scintillation counter (Tri-Carb 3110 TR, PerkinElmer, Inc.).

In Situ Closed Loop Study in Rats In situ closed loop study was conducted as previously reported.23–25 Briefly, after a minimal abdominal incision was made under light ether anesthetization and the contents within the gastrointestinal (GI) tract were sufficiently washed, a 5-cm long duodenum, jejunum or ileum loop was closed by ligation approximately 2 cm distal to both ends of each intestinal section. Special care was taken to avoid damaging blood vessels and to include as much of a complete mesenteric blood vessel arch as possible for each loop. After injection of 0.2 mL adefovir dipivoxil solution (1 mM adefovir dipivoxil in double-distilled water) into each loop by using 1 mL syringe with 31-gauge needle, the whole GI tract was carefully replaced in the abdominal cavity. The incision was then closed using clamps and kept moist by covering with gauze pads presoaked with normal saline. The rat was kept warmed by a lamp. At 60 min after drug injection, each loop was removed, transferred into a beaker containing 50 mL of methanol and cut into small pieces using scissors to facilitate the extraction of adefovir dipivoxil. After manual shaking and stirring with a glass rod for 1 min, a 100-µL aliquot of the supernatant was collected from each beaker and stored at −80°C until the HPLC analysis of adefovir dipivoxil.

In Vivo Tissue Distribution Study in Rats [3H]Adefovir dipivoxil (dissolved in 0.9% NaCl injectable solution) was administered orally to control or 1,25(OH)2D3-treated rats (n=4–5 in each group) at a dose of 36.6 µmol/kg as adefovir. At 60 min after oral dosing, 300 µL of blood was collected, and approximately 1 g each of the brain, liver, heart, kidney, small intestine, and muscle was excised. A 50-µL plasma sample was obtained by centrifugation of the blood sample at 16000×g at 4°C for 10 min. The radioactivity of [3H]adefovir in the plasma sample was measured by liquid scintillation counter. For tissue samples, each tissue was homogenized (Ultra-Turax T25, Janke&Kunkel, IKA-Labortechnik, Staufen, Germany) with four volumes of 0.9% NaCl injectable solution. After centrifugation for 10 min at 3000 rpm, a 100-µL aliquot of the supernatant was analyzed for radioactivity by liquid scintillation counter.

HPLC Analysis The concentration of adefovir dipivoxil in the loop samples was determined as reported previously with slight modification.20 A 100-µL aliquot of the samples was deproteinized with a 200-µL aliquot of acetonitrile. After vortex-mixing and centrifugation at 16000×g for 10 min, a 50-µL aliquot of the supernatant was directly injected into a reversed phase (C18) HPLC column. The mobile phase was a mixture of 10 mM KH2PO4 (pH 4.0) and acetonitrile (60:40, v/v) with 0.1% triethylamine (TEA) (v/v). The flow rate was 1 mL/min. The column effluent was monitored by a UV detector set at 262 nm. The retention time was approximately 8 min, and the limit of quantitation was 100 ng/mL. The inter- and intra-day coefficients of variation were below 11.3%.
Data Analysis  Non-compartmental analysis (WinNonlin, version 3.1, NCA200 and 201; Pharsight Co.) was conducted to calculate the following pharmacokinetic parameters: the total area under the plasma concentration–time curve from zero time to time infinity (AUC); the time-averaged total body clearance (CL); the terminal half-life (t1/2); the apparent volume of distribution at steady state (Vss); and the mean residence time (MRT). For comparison, the extent of absolute oral bioavailability (F; expressed as percent of dose administered) was calculated by dividing the dose-normalized AUC after oral administration by the dose-normalized AUC after intravenous injection.\(^{27}\) The peak plasma concentration (Cmax) and time to reach Cmax (Tmax) were read directly from the experimental data. The tissue-to-plasma partition coefficient (Kt) was calculated by dividing the tissue drug concentration by the plasma drug concentration.\(^{28}\)

Statistical Analysis  A p-value of less than 0.05 was considered statistically significant using a t-test between two means for unpaired data or a Duncan’s multiple range test post hoc ANOVA among three means for unpaired data. All data were expressed as mean±standard deviation, except for median (ranges) for Tmax, and were rounded to three significant figures.

RESULTS

In Vivo Intravenous Pharmacokinetic Study in Rats  Prior to the in vivo intravenous pharmacokinetic study of adefovir, preliminary studies regarding body weight, serum and urine chemistry analysis and CL\text{ir} were conducted. As a result, there were no differences (p>0.05) in total plasma protein, plasma albumin, blood urea nitrogen, SGOT, sGPT and CL\text{ir}, indicating that liver and kidney function are normal in 1,25(OH)\textsubscript{2}D\textsubscript{3}-treated rats, compared to control rats (data not shown). However, it is interesting to note that body weight gain was significantly (p<0.05) smaller in 1,25(OH)\textsubscript{2}D\textsubscript{3}-treated rats than in control rats, which was also consistent with our previous reports by Kim et al.\(^{29}\) Effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} on the pharmacokinetics of intravenous adefovir itself in rats were first evaluated. Plasma concentration–time profiles of adefovir after its intravenous administration at a dose of 15 µmol/kg in control and 1,25(OH)\textsubscript{2}D\textsubscript{3}-treated rats are shown in Fig. 1. Relevant pharmacokinetic parameters are listed in Table 1. Plasma concentrations of adefovir declined in a multi-exponential manner with a terminal half-life of 209–256 min in both groups (Fig. 1). There was no significant difference in AUC, CL, V\text{ss}, MRT, and t1/2 of adefovir between control and 1,25(OH)\textsubscript{2}D\textsubscript{3}-treated rats (Table 1).

In Vivo Oral Pharmacokinetic Study in Rats  Effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} on the pharmacokinetics of oral adefovir dipivoxil in rats were then evaluated. In the preliminary studies, radio-HPLC analysis demonstrated that the level of adefovir dipivoxil was negligible in rat plasma from oral pharmacokinetic study of [\textsuperscript{3}H]adefovir dipivoxil, indicating that adefovir was found to be the only species detected (data not shown). Plasma concentration–time profiles of adefovir after the oral administration of adefovir dipivoxil at a dose of 36.6 µmol/kg (as equivalent dose of adefovir, 36.6 µmol/kg) in control (●) and 1,25(OH)\textsubscript{2}D\textsubscript{3}-treated (○) rats are shown in Fig. 2, and relevant pharmacokinetic parameters are listed in Table 2. The plasma concentrations of adefovir increased during 60–90 min after dosing and then decreased in a mono-exponential manner in

<table>
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<tr>
<th>Parameter</th>
<th>Control</th>
<th>1,25(OH)\textsubscript{2}D\textsubscript{3}</th>
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<tbody>
<tr>
<td>AUC (×10\textsuperscript{3} µmol/min)</td>
<td>0.611±0.244</td>
<td>0.825±0.233</td>
</tr>
<tr>
<td>CL (mL/min/kg)</td>
<td>27.1±9.6</td>
<td>19.3±6.0</td>
</tr>
<tr>
<td>V\text{ss} (×10\textsuperscript{3} mL/kg)</td>
<td>7.00±2.68</td>
<td>6.21±1.40</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>266±70</td>
<td>328±41</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>209±48</td>
<td>256±22</td>
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both rats (Fig. 2). Importantly, the \( AUC \) and \( C_{\text{max}} \) of adefovir were significantly \( (p<0.05) \) higher in 1,25(OH)\(_2\)D\(_3\)-treated rats than in control rats by 64.5 and 109.9%, respectively (Table 2). As a result, \( F \) increased significantly \( (p<0.05) \) in 1,25(OH)\(_2\)D\(_3\)-treated rats vs. control rats by 21.5% (Table 2).

### In Vivo Tissue Distribution Study in Rats

Effects of 1,25(OH)\(_2\)D\(_3\) on the intestinal absorption of adefovir dipivoxil, the P-gp substrate, in rat duodenum, jejunum and ileum segments were evaluated. Fractions of adefovir dipivoxil remaining at 60 min after its injection into the duodenum, jejunum and ileum loops of control and 1,25(OH)\(_2\)D\(_3\)-treated rats are shown in Fig. 3. Significant amounts (18.7–29.8% of dose injected) of adefovir dipivoxil remaining were observed in all the loops tested. However, there was no significant difference in the remaining fraction of adefovir dipivoxil in all the intestinal loops between control and 1,25(OH)\(_2\)D\(_3\)-treated rats. Moreover, in both groups, no significant difference in the remaining fraction of adefovir dipivoxil was observed among duodenum, jejunum and ileum loops.

**DISCUSSION**

The present study was conducted to investigate the effect of 1,25(OH)\(_2\)D\(_3\), an active form of vitamin D, on the oral absorption and disposition of adefovir dipivoxil, a P-gp substrate and its major active metabolite, adefovir, a MRP4/Mrp4 substrate. First, we studied the effects of 1,25(OH)\(_2\)D\(_3\) on the pharmacokinetics of intravenous adefovir in rats (Fig. 1, Table 1). Orally-administered adefovir dipivoxil is rapidly converted into adefovir by esterase located in enterocytes and reaches the systemic circulation as a form of adefovir.\(^{13}\) Thus, the intravenous pharmacokinetic study was conducted with adefovir itself, instead of with adefovir dipivoxil, in the present study. The major elimination route of adefovir is renal excretion.\(^{18,19}\)

As shown in Table 1, the \( CL \) of adefovir did not change significantly with 1,25(OH)\(_2\)D\(_3\) treatment, which suggests that renal excretion of intravenous adefovir may not be significantly influenced by 1,25(OH)\(_2\)D\(_3\).

Then, the effects of 1,25(OH)\(_2\)D\(_3\) on the pharmacokinetics of oral adefovir dipivoxil were evaluated (Fig. 2, Table 2). As shown in Table 2, the \( AUC \) and \( C_{\text{max}} \) of adefovir, the final active metabolite, after the oral administration of adefovir dipivoxil in rats increased significantly with 1,25(OH)\(_2\)D\(_3\) treatment, which suggests that the oral absorption of adefovir dipivoxil and/or adefovir is enhanced by 1,25(OH)\(_2\)D\(_3\). In the intestine, adefovir dipivoxil can diffuse through the apical membrane of enterocytes and is rapidly hydrolyzed to adefovir. Then, the intracellularly generated adefovir is absorbed into the blood by crossing the basolateral membrane of enterocytes primarily via MRP4/Mrp4-mediated efflux rather than by passive diffusion, due to the highly negative charge of adefovir.\(^{13,15,17}\)

Consistent with this, our previous *in vitro* study using everted

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**Table 2. Pharmacokinetic Parameters of Adefovir after the Oral Administration of Adefovir Dipivoxil at a Dose of 36.6\(\mu\)mol/kg (as Equivalent Dose of Adefovir, 36.6\(\mu\)mol/kg) in Control and 1,25(OH)\(_2\)D\(_3\)-Treated Rats (n=3–4)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1,25(OH)(_2)D(_3)</th>
</tr>
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<tbody>
<tr>
<td>( AUC \times 10^3 \mu\text{mol} \cdot \text{min}^{-1} )</td>
<td>0.693±0.172</td>
<td>1.14±0.24*</td>
</tr>
<tr>
<td>( C_{\text{max}} ) ((\mu)mol)</td>
<td>1.72±0.40</td>
<td>3.61±1.16*</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (min)</td>
<td>60 (60–120)</td>
<td>90 (60–120)</td>
</tr>
<tr>
<td>( F ) (%)</td>
<td>46.5</td>
<td>56.5</td>
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\(^*\) Significantly different from the control group \((p<0.05)\).
rat intestinal sac preparations showed that rat intestinal Mrp4 is functionally induced by 1,25(OH)2D3 via VDR activation. Moreover, Chow et al. has reported that the mRNA expression of rat intestinal Mrp4 remains unchanged while the protein expression is induced by the same 1,25(OH)2D3 treatment procedure as this study. Thus, the enhanced oral absorption of adefovir dipivoxil observed in 1,25(OH)2D3-treated rats could be attributed partly to the induction of Mrp4 function by 1,25(OH)2D3 treatment via VDR activation.

Since adefovir dipivoxil is a substrate of P-gp, and its intestinal permeation can be limited by P-gp-mediated efflux, we could not rule out the regulation of P-gp function by 1,25(OH)2D3 treatment. However, when the effects of 1,25(OH)2D3 on the intestinal absorption of adefovir dipivoxil in rat duodenum, jejunum and ileum segments were observed using the in situ closed loop technique (Fig. 3), the results suggest that the intestinal permeation of adefovir dipivoxil, the P-gp substrate, remained unchanged by 1,25(OH)2D3 treatment. Consistent with this, our previous in vitro study using everted rat intestinal sac preparations has indicated that the function of rat intestinal P-gp (i.e., digoxin efflux) is not influenced significantly by 1,25(OH)2D3 treatment, accompanying with no change of Mdr1 expression. Thus, the unchanged intestinal permeation of adefovir dipivoxil between the two groups could be partly explained by the unaltered P-gp function with 1,25(OH)2D3 treatment.

The oral absorption (bioavailability) of a drug depends on both intestinal membrane permeation and intestinal/hepatic first-pass elimination of the drug. Adefovir is mainly eliminated by renal excretion, and it is not a substrate of CYP isozymes. Moreover, there was no significant difference in the pharmacokinetic parameters (including CL) of intravenous adefovir between the control and 1,25(OH)2D3-treated rats (Table 1). This result indicates that the overall disposition (including intestinal and hepatic elimination) of adefovir was not significantly changed by 1,25(OH)2D3 treatment in rats. Thus, it is plausible that the increased oral absorption of adefovir after the oral dosing of adefovir dipivoxil in 1,25(OH)2D3-treated rats (Table 2) could be attributed mainly to the change in the intestinal membrane permeation (i.e., apical membrane transport of adefovir dipivoxil and basolateral membrane transport of adefovir), rather than intestinal/hepatic first-pass elimination of adefovir. Moreover, the result shown in Fig. 3 suggests that the apical membrane transport of adefovir dipivoxil in enterocytes was not significantly changed by 1,25(OH)2D3 treatment in rats. Thus, it is inferred that Mrp4-mediated basolateral membrane transport of adefovir in enterocytes could be significantly involved in the enhancement of oral absorption of adefovir by 1,25(OH)2D3 treatment. However, further studies are definitely necessary to determine whether other factors might affect the enhanced absorption of adefovir by 1,25(OH)2D3 treatment.

The effects of 1,25(OH)2D3 on the tissue distribution of adefovir after the oral administration of adefovir dipivoxil in rats were also evaluated (Fig. 4). Notably, 1,25(OH)2D3 treatment significantly reduced the Kp of adefovir in the intestine by 84.3%. Considering that plasma concentrations of adefovir at 60min after oral administration of adefovir dipivoxil increased in 1,25(OH)2D3-treated rats by 144%, this result likely indicates that the intestinal accumulation of adefovir can be reduced considerably by 1,25(OH)2D3 treatment. Intracellular adefovir can be absorbed into the blood via basolateral Mrp4-mediated efflux in enterocytes. Thus, the reduced intestinal accumulation of oral adefovir could be attributed to an increase in the basolateral efflux of intracellular adefovir into blood, likely due to induced Mrp4 function by 1,25(OH)2D3 treatment, which corresponds well with those found in the present oral pharmacokinetic study of adefovir dipivoxil in rats (Fig. 2, Table 2).

In this study, the oral absorption of adefovir dipivoxil was enhanced by 1,25(OH)2D3-treatment, without change in the systemic pharmacokinetics of adefovir. From a point of view of clinical relevance, osteomalacia, which is manifested as bone pain and may contribute to fractures, is regarded as an important side-effect of adefovir therapy. Since 1,25(OH)2D3 (calcitriol) is prescribed for the treatment of osteomalacia, the concurrent use of 1,25(OH)2D3 could be prevalent in adefovir therapy. Indeed, the clinical use of 1,25(OH)2D3 for the treatment of osteomalacia induced by adefovir therapy has also been reported. Thus, the present in vivo results could lead to further studies in clinically significant transporter-mediated drug interactions with 1,25(OH)2D3, such as the 1,25(OH)2D3–adefovir interaction. With respect to the drug interaction with 1,25(OH)2D3, an active form of vitamin D in vivo, only a few pharmacokinetic studies have been reported for clinically important drugs so far. For example, Chow et al. demonstrated enhanced renal secretory intrinsic clearance of digoxin in 1,25(OH)2D3-treated mice due to increased kidney P-gp function. More recently, Kim et al. reported that the systemic pharmacokinetics of two cephalosporins, cefdinir and cefadroxil, were dramatically changed, likely due to decreased renal excretion in 1,25(OH)2D3-treated rats.

In conclusion, the present study indicates that 1,25(OH)2D3 treatment can enhance the oral absorption of adefovir dipivoxil (i.e., increased AUC of adefovir, a Mrp4 substrate) via the induction of basolateral Mrp4 function in rat intestine. However, the impact of 1,25(OH)2D3 treatment on the pharmacokinetics of intravenous adefovir is quite limited. To the best of our knowledge, this study provides the first reported data regarding the effect of 1,25(OH)2D3 on the in vivo fates of adefovir dipivoxil and adefovir after their intravenous and oral dosing in rats. Further studies of clinically significant P-gp and/or Mrp4-mediated 1,25(OH)2D3–drug interactions is required in vivo.

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Conflict of Interest The authors declare no conflict of interest.

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