Pharmacokinetics, safety and tolerance of single- and multiple-dose adefovir dipivoxil in healthy Chinese subjects

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Aims

To assess the pharmacokinetics, safety and tolerance of single- and multiple-dose adefovir dipivoxil (ADV) in healthy Chinese subjects.

Methods

Forty-two healthy subjects were randomized into 5, 10, 20, 40 and 60-mg dose groups for safety assessment. Nine and 10 healthy males were enrolled for a single-dose pharmacokinetic profile and assessment of the effect of food on the pharmacokinetics of adefovir (PMEA), respectively. Another 10 healthy subjects were enrolled for a multiple-dose safety assessment and pharmacokinetic profile. Safety and tolerance were evaluated by monitoring adverse events and laboratory parameters, and pharmacokinetics were assessed by determining PMEA concentrations with a validated LC-MS/MS method.

Results

No serious adverse events occurred. The pharmacokinetic parameters of PMEA following ADV 10, 20 and 40 mg were: geometric mean [95% confidence interval (CI)] for $AUC_{0-24\text{ h}}$ 227 (205, 253), 423 (361, 506) and 686 (585, 828) $\mu\text{g l}^{-1}\text{ h}$, C_{max} 23.0 (20.7, 27.3), 47.4 (42.8, 53.2) and 83.6 (72.6, 97.4) $\mu\text{g l}^{-1}$, arithmetic mean (95% CI) for $t_{1/2}$ 6.8 (6.3, 7.3), 7.4 (6.7, 8.1) and 7.7 (6.5, 8.9) h, median value (range) for t_{max} 1.00 (1.00–2.00), 0.75 (0.75–2.50) and 1.00 (0.75–2.00) h, respectively. The steady-state pharmacokinetics parameters were similar to those following a single dose. The AUC of PMEA was unaffected by food.

Conclusion

ADV is safe and well tolerated in healthy Chinese subjects. The mean C_{max} of PMEA is proportional to dose, but the linearity of AUC needs further study. There is no accumulation following multiple doses of ADV and the extent of absorption of PMEA is unaffected by food.

Introduction

Adefovir [9-(2-phosphonylmethoxyethyl) adenine] (PMEA) (Figure 1), a phosphonic acid derivative of adenine, has broad-spectrum activity against human immunodeficiency virus (HIV), herpes virus, hepatitis B virus

(HBV) and adenoviruses [1]. In order to improve the limited oral bioavailability, the bis-pivaloyloxymethyl ester of adefovir [bis-POM-PMEA, adefovir dipivoxil (ADV), Figure 1] has been used for chronic hepatitis B in adults with evidence of HBV replication. ADV can

Figure 1

Chemical structure of adefovir dipivoxil (ADV) and phosphonylmethoxyethyl adenine (PMEA)

be rapidly hydrolysed to PMEA in the gastrointestinal tract after oral administration [2], which is transported into cells (with a long intracellular half-life of 12–36 h) and converted to active PMEA diphosphate through phosphorylation [3]. PMEA diphosphate inhibits HBV polymerase by competitively binding endogenous substrate [deoxyadenosine triphosphate (dATP)] and results in chain termination of DNA synthesis after incorporation into viral DNA [4].

Previous studies [5] have indicated that the maximum observed PMEA plasma concentrations ($C_{\max}$) are rapidly achieved (0.76–0.1.75 h, median value) and then decline with a well-characterized median terminal elimination half-life ($t_{1/2}$) of 6–7 h, and the absolute oral bioavailability and urinary recovery of PMEA are approximately 59% and 45.3% (median value), respectively, following a single oral dose of ADV 10 mg to healthy subjects or patients with chronic hepatitis B; there is no apparent accumulation of PMEA at steady state following 7 days of once-daily dosing of ADV 10 mg in patients with chronic hepatitis B. PMEA is eliminated by the kidney as unchanged drug through a combination of tubular secretion and glomerular filtration and >98% of an intravenous dose is recovered in urine over 24 h [6]. Intracellular activation of a small fraction (<10%) of the dose by cellular kinases leads to prolonged antiviral effects, which is not easily predicted from conventional pharmacokinetic studies [7].

Current estimates indicate that 300–400 million people worldwide, and approximately 120 million in China, are chronically infected with HBV, and >300 000 patients in China die from chronic hepatitis B each year [8]. Interferon (IFN)- α and lamivudine are licensed for chronic hepatitis B, but clinically limited, because of the tolerability and limited efficacy in some subpopulations for IFN- α and resistance for lamivudine. ADV is reported to be more effective against HBV without resistance and expected to be much more widely used in China than in any other country.

A new crystal form of ADV has been developed and approved for Phase I clinical trial by the State Food and Drug Administration (SFDA) of China. It is well known that different crystal forms of a drug may lead to changes in pharmacokinetic or pharmacodynamic behaviour. The objectives of this study were to assess the pharmacokinetics, safety and tolerance of the new crystal form of ADV in healthy Chinese subjects, to provide evidence for the next clinical trials and to validate its conventional oral dosage regimen.

Materials and methods

The study was performed at the Institute of Clinical Pharmacology and approved by the ethics committee, Qilu Hospital of Shandong University, and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Subjects

All subjects (Han nationality) in this study were young students from Shandong University and enrolled according to the following criteria: (i) body mass index (BMI) of 19–24 kg m⁻²; (ii) no pregnancy or menstruation for female subjects; (iii) no smoking, drinking or drug addiction before or during the study; (iv) normal vital signs, physical and laboratory findings, abdominal ultrasonography and chest fluoroscopy; (v) no history of allergy to any components of ADV tablets, or history of nervous or psychological disease; (vi) no drugs or other medication, or any kind of clinical trial that might interfere with absorption, distribution, metabolism or excretion of ADV or be harmful to human organs 3 months before or during the study; and (vii) voluntary signature of an informed consent form.

Subjects with systemic disease or addiction to alcohol, cigarette or psychic drugs, or with laboratory abnormalities, or positive hepatitis B surface antigen, hepatitis A virus, hepatitis C virus antibody tests, were excluded.

Drugs

ADV tablets, 5 mg (batch no. 0403001) and 10 mg (batch no. 030127), were from Qilu Pharmaceutical Co. (Jinan, Shandong Province, China) and manufactured according to Good Manufacturing Practice.

Study design

Single-dose study A randomized, open-label, dose-escalating study was conducted to assess ADV safety and tolerance. Forty-two healthy subjects, half male and half female, were randomized into 5, 10, 20, 40, and 60-

mg dose groups (6–10 subjects in each) balanced by sex and body weight. The trial was designed to begin with the 5-mg dose group and would not proceed to the higher dose group until the safety and tolerance of the lower were confirmed. The subjects were required to fast overnight (10 h) before and for 4 h after administration and water intake was allowed 2 h after administration.

Nine healthy male subjects were randomized into 10-, 20- and 40-mg dose groups for the pharmacokinetic profile, according to a triple-period crossover design with a 1-week wash-out period. Each subject received the scheduled dosage with 250 ml of water, after an overnight fasting (10 h). Water intake was allowed 2 h postdose and low-fat standard meals were provided at 4 h and 10 h postdose. Blood samples (4 ml) were collected at 0 (predose), 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h postdose. Serum was separated, decanted, frozen and stored at -80°C for analysis. Urine samples were collected prior to dosing and over the intervals 0–2, 2–4, 4–6, 6–8, 8–10, 10–12 and 12–24 h postdose. The total volume of urine in each interval was recorded, 10 ml of which was centrifuged, decanted, frozen and stored at -80°C for analysis.

Another 10 healthy male subjects were randomized into fasting and dining groups to assess the effect of food on the pharmacokinetics of PMEA, according to a double-period crossover design with 1 week's wash-out period. All subjects were required to fast overnight (10 h). One group received 10 mg of ADV with 250 ml of water in a fasting condition and food was allowed 4 h postdose; another group received 10 mg of ADV with 250 ml of water 5 min postmeal (approximately 1000 kcal high-fat meal). Water intake was allowed 2 h postdose and low-fat standard meals were provided at 4 h and 10 h postdose in both groups. Blood samples were collected in the same way as in the single-dose pharmacokinetic study.

Multiple-dose study Ten healthy subjects, half male and half female, were given 10 mg of ADV orally once daily for 7 days to assess its safety, tolerability and pharmacokinetics. Intake of food and water was the same as in the single-dose study. Blood samples (4 ml) were collected prior to dosing on days 1, 5, 6 and 7 (0 h prior to dosing, 24 h postdose on days 4, 5 and 6) and 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h postdose on day 1 and day 7. Serum was separated, decanted, frozen and stored at -80°C for analysis.

Safety and tolerance

All subjects were kept in the study unit and continuously observed. Details of symptoms such as asthenia, head-

ache, dizziness, nausea, vomiting, diarrhoea and abdominal pain were obtained through a daily questionnaire and recorded by the study physicians. Safety assessments such as physical examination, vital signs, electrocardiogram, routine blood and urine test, thrombotest and blood biochemical test were conducted 24–48 h before and 24 h after administration in the single-dose study, 24–48 h before and on days 2, 4 and 8 after administration in the multiple-dose study. The blood biochemical test included potassium (K^{+}), sodium (Na^{+}), chlorine (Cl^{-}), calcium (Ca^{2+}), phosphorus (P), urea nitrogen (BUN), creatinine (Cr), total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartic transaminase (AST), γ -glutamyltransferase (GGT), alkaline phosphatase (AKP), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), fasting blood glucose (GLU), serum amylase (AMY), creatine kinase (CK), lactate dehydrogenase (LDH), triglyceride (TG) and total cholesterol (CHOL).

Adverse events which occurred during the study, defined as mild (awareness of a sign or symptom but tolerated), moderate (discomfort which may interfere with daily activities) or serious (death, life-threatening, requiring hospitalization or incapacitating) were recorded and reported according to Good Clinical Practice. The causality between the study drug and an adverse event, described as 'certainly', 'probably', 'possibly', 'suspected' or 'not related', was verified.

Drug analysis

Drug analysis was performed in the Drug Analysis Centre of Shandong University (Jinan, China). The concentrations of PMEA in serum and urine were determined by a validated LC-MS/MS method. Acyclovir was selected as the internal standard. The serum sample (0.5 ml) was pretreated by precipitating protein with methanol and the supernatant was evaporated to dryness and reconstituted with 200 μl of mobile phase before it was injected for analysis. The urine sample was directly injected for analysis after dilution with mobile phase. The analyte was separated on a Diamonsil C_{18} column (250×4.6 mm i.d., 5 μm ; Beijing Dikma Co., Beijing, China) by isocratic elution with methanol:water:formic acid (20 : 80 : 0.1 v/v/v) at a flow rate of 0.6 ml min^{-1} and analysed by an API 4000 triple-quadrupole mass spectrometric instrument (Applied Biosystems, Foster City, CA, USA) in multiple reaction monitoring mode. The precursors to product ion transitions of m/z 274 $\rightarrow$ 162 and m/z 226 $\rightarrow$ 135 were used to measure and quantify PMEA and internal standard, respectively. The analysis of blank serum and urine indicated no interference of endogenous components with PMEA in final

extract. The weighted ($1/x^2$) calibration curve was linear over the serum concentration range of 1.25–160.00 ng ml⁻¹ and urine concentration range of 0.05–8.00 µg ml⁻¹, with a correlation coefficient (r) of 0.9992 and 0.9978, respectively. The lower limit of quantification for PMEA was 1.25 ng ml⁻¹. The mean inter- and intraday precision (RSD) was <10%, the mean method recoveries were 96.3–102.0% and the mean extraction recoveries from serum were 56.5–59.3%. The samples were determined once, but those in doubt were re-analysed.

Pharmacokinetic calculations

The mean PMEA concentrations in serum at each time point and in urine over each interval were determined by averaging data and the pharmacokinetic parameters were calculated by Drug and Statistic (DAS) software (version 1.0; Rui-yuan Sun, Wuhu, Anhui Province, China). The $C_{\max}$ and $t_{\max}$ were observed values, the area under the concentration–time curve (AUC) was calculated according to the linear trapezoidal rule. The mean steady state concentration ($\bar{C}_{ss}$) and the degree of fluctuation (DF%) was calculated as $AUC_{0-\tau}/\tau$ and $(C_{\max}^{ss} - C_{\min}^{ss})/\bar{C}_{ss} \times 100\%$, respectively.

Statistical analysis

The statistical software of Statistical Analysis System v. 8.1 (SAS 8.1; SAS Institute Inc., Cary, NC, USA) was used to perform statistical analysis. Geometric mean and associated 95% confidence intervals (CIs) for AUC and $C_{\max}$ (CIs were first determined using logarithms of individual values and then expressed as linear values), arithmetic mean and associated 95% CIs for apparent volume of distribution (V_d/F), clearance (CL/F), mean residence time (MRT), $t_{1/2}$ and urinary excretion rate, median value and associated range for $t_{\max}$ were estimated for pharmacokinetic parameters, respectively. The coefficient of variation (CV) was calculated to express the variability of the pharmacokinetic parameters $[(SD/mean) \times 100]$. Statistical comparisons between pharmacokinetic parameters of single and multiple doses were performed by a paired t -test and the variances of pharmacokinetic parameters between fasting and dining groups, male and female subjects were compared by analysis of variance (ANOVA), respectively. A P -value of ≤ 0.05 was considered significant.

The analysis of safety was also performed with SAS software v. 8.1. The variations of laboratory parameters before and after administration in single- and multiple-dose studies were analysed by paired t -test and the variances among groups in the single-dose study were analysed by ANOVA. Adverse events were summarized with frequencies and percentages.

Results

Study population

Characteristics of the study population are presented in Table 1. There were no significant differences ($P > 0.05$, by ANOVA) in age, weight or height among dose groups in the single-dose study. No subject dropped out of the study.

Safety and tolerance

ADV was well tolerated, no serious adverse events occurred during the study and all subjects were in good compliance. The adverse reactions, including nausea, abdominal pain and diarrhoea were found in both single- and multiple-dose studies; in addition, asthenia was frequently observed in the multiple-dose study. All subjects that suffered from adverse events were female, except for one male suffering from diarrhoea in the 20-mg dose group. Whether the adverse reactions were gender-related is not known and needs further investigation in a large sample study.

In the single-dose study, high ALT concentrations were found in two subjects in the 20-mg group and TBIL, DBIL and IBIL increases were also observed in different dose groups. In the multiple-dose study, increases in ALT concentrations were also found in one subject on day 2 and day 4 after oral administration but returned to normal on day 8 (24 h after the last administration) without treatment. The subject was examined carefully by the study physicians and received further consultation and examination 1 week after the study. In addition, increases in CK and LDH concentrations were observed in one subject on day 8 and another subject on day 4, respectively.

No changes of clinical significance were found in routine urine and blood tests. Statistically significant ($P < 0.05$, by t -test) differences from baseline in ALB, CHOL, GLU and AMY in different dose groups, and notable differences ($P < 0.05$, by ANOVA) among dose groups in Na⁺, Cl⁻, Ca²⁺ and TP were found in the single-dose study, but none was beyond the normal range. Notable increases in GGT, AKP and Cr and prolongation of prothrombin time (PT) were observed on different days following multiple doses, but they were all within normal range.

All adverse events and laboratory abnormalities were mild and tolerable, did not lead to discontinuation of the study; recovery was without treatment.

Pharmacokinetic profile

Single-dose pharmacokinetic study A one-compartment first-order absorption model was fit to mean concentra-

Table 1
Characteristics of study population [mean $\pm$ SD (range)]

Group <i>N</i>	5-mg group 6		10-mg group 10		Safety assessment 20-mg group 10		Single-dose 40-mg group 10		60-mg group 6		Pharmacokinetic profile Single-dose 9		Effect of food 10		Multiple-dose 10-mg group 10	
Sex (female/ male)	3/3		5/5		5/5		5/5		3/3		0/9		0/10		5/5	
Age (years)	22.3 ± 1.4 (21–24)		22.3 ± 1.6 (20–24)		22.7 ± 1.5 (21–26)		23.1 ± 1.9 (19–25)		21.4 ± 1.7 (20–24)		23.1 ± 1.5 (21–25)		22.6 ± 1.4 (20–24)		21.8 ± 0.9 (21–23)	
Height (cm)	166 ± 8 (158–176)		171 ± 9 (161–186)		166 ± 8 (155–177)		168 ± 6 (160–180)		170 ± 8 (160–182)		173 ± 4 (170–182)		175 ± 5 (170–186)		166 ± 7 (158–174)	
Weight (kg)	59 ± 11 (50–72)		64 ± 8 (58–82.5)		58 ± 8 (50–70)		58 ± 9 (49–80)		60 ± 10 (50–74)		65 ± 7 (57–80)		68 ± 7 (60–82.5)		58 ± 9 (50–76)	
BMI (kg m ⁻²)	21.1 ± 2.3 (19–23)		22.1 ± 1.2 (20–24)		20.8 ± 1.6 (19–24)		20.4 ± 1.9 (19–24)		20.8 ± 1.4 (19–23)		21.6 ± 1.8 (20–24)		22.1 ± 1.4 (20–24)		21.2 ± 1.8 (19–24)	

F, Female; *M*, male; BMI, body mass index.

F, Female; M, male; BMI, body mass index.

tion data by using maximum likelihood estimation, following an oral dose of ADV 10, 20 or 40 mg. The main pharmacokinetic parameters of PMEA are presented in Table 2; the serum concentration–time curves and cumulative urinary excretion–time curves of PMEA are shown in Figures 2 and 3, respectively. The arithmetic mean (95% CI) of the urinary excretion rate was 34.3 (31.4, 36.6), 36.2 (29.5, 42.5) and 35.0 (25.8, 44.1)% and the excretion constant k_e was 0.055 (0.037, 0.073), 0.066 (0.056, 0.076) and 0.060 (0.034, 0.086) h⁻¹, respectively.

The linearity between mean $C_{\max}$, $AUC_{0,\infty}$ and dose was evaluated by linear regression analysis, with a regression equation $Y = 2.032X + 4.845$ ($r = 0.9976$) and $Y = 15.586X + 92.89$ ($r = 0.9943$), respectively. Further statistical analysis (two tailed t -test) on r indicated that the mean $C_{\max}$ was linear, but $AUC_{0,\infty}$ was not. Analysis of individuals indicated that the $AUC_{0,\infty}$ was nonlinear in four of nine subjects and linear in the others. The reasons for the nonlinearity are not clear. One possibility is that we administered only three doses and variability in the AUC at one dose level would therefore have a large effect on the overall trend (the $AUC_{0,\infty}$ at 40 mg was only three times that at 10 mg). Another possibility is that only nine subjects had been studied for the pharmacokinetic profile and the variability in AUC in one or more individual would affect the overall result greatly. Of note, the V_d/F , CL/F , MRT , $t_{1/2}$ and urinary excretion rates of PMEA were similar ($P > 0.05$) at the three dose levels.

Effect of food on PMEA pharmacokinetics The main pharmacokinetic parameters of PMEA in the fasting and nonfasting states are presented in Table 3 and the mean concentration–time curves of PMEA in Figure 4. No statistical significance of the pharmacokinetic parameters was found in the fasting or nonfasting groups by ANOVA, except for V_d ($P < 0.05$) and $t_{\max}$ ($P < 0.01$), indicating that food did not affect the extent but the rate of absorption of PMEA after oral dosing.

Multiple-dose pharmacokinetic study The mean $C_{\min}$ on days 1, 4, 5, 6 and 7 was 2.22, 2.09, 2.15, 2.12 and 2.32 $\mu\text{g l}^{-1}$, respectively, which was similar ($P > 0.05$) following multiple doses of ADV 10 mg continuously for 7 days. The main pharmacokinetic parameters of PMEA on day 1 and day 7 are presented in Table 4 and the mean PMEA serum concentration–time curves in Figure 5. No significant variance ($P > 0.05$) was found between the pharmacokinetic parameters on day 1 and day 7 by a paired t -test, indicating that the steady-state pharmacokinetic parameters of PMEA were similar to

Table 2Main pharmacokinetic parameters of PMEA following single-dose of ADV ($n = 9$)

Parameter/summary statistic	10-mg group	20-mg group	40-mg group
$AUC_{0-24\text{ h}}$ ($\mu\text{g l}^{-1}\text{ h}$)			
Geometric mean (95% CI) CV (%)	227 (205, 253) 24	423 (361, 506) 26	686 (585, 828) 26
$AUC_{0-\infty}$ ($\mu\text{g l}^{-1}\text{ h}$)			
Geometric mean (95% CI) CV (%)	230 (209, 257) 16	427 (365, 510) 25	692 (591, 833) 26
C_{max} ($\mu\text{g l}^{-1}$)			
Geometric mean (95% CI) CV (%)	23.0 (20.7, 27.3) 21	47.4 (42.8, 53.2) 17	83.6 (72.6, 97.4) 22
V_d/F (ml kg^{-1})			
Arithmetic mean (95% CI) CV (%)	7.0 (5.7, 8.3) 29	7.4 (5.7, 9.1) 35	8.1 (7.0, 9.1) 20
CL/F ($\text{ml h}^{-1}\text{ kg}^{-1}$)			
Arithmetic mean (95% CI) CV (%)	0.63 (0.54, 0.71) 21	0.68 (0.60, 0.76) 18	0.91 (0.72, 1.10) 32
MRT (h)			
Arithmetic mean (95% CI) CV (%)	7.8 (7.3, 8.3) 10	7.7 (7.4, 8.0) 5	7.7 (7.3, 8.1) 8
$t_{1/2}$ (h)			
Arithmetic mean (95% CI) CV (%)	6.8 (6.3, 7.3) 12	7.4 (6.7, 8.1) 15	7.7 (6.5, 8.9) 23
t_{max} (h)			
Median (range)	1.00 (1.00–2.00)	0.75 (0.75–2.50)	1.00 (0.75–2.00)

Figure 2

Mean serum concentration–time curves of PMEA following a single dose of ADV ($n = 9$). 10 mg (◆); 20 mg (■); 40 mg (▲)

Figure 3

Mean cumulative urinary excretion–time curves of PMEA following a single dose of ADV ($n = 9$). 10 mg (◆); 20 mg (■); 40 mg (▲)

those following a single dose and no accumulation was found following multiple doses of ADV.

Compared with those in males, lower V_d , prolonged $t_{1/2}$ and MRT and higher C_{max} were observed in female subjects, which may be caused by the body weight differences, but no statistical significance ($P > 0.05$) was found.

Discussion

Evidence of renal toxicity has been noted in all species in preclinical studies and nephrotoxicity was the most important dose-limiting toxicity of ADV at daily doses

of 30–120 mg in clinical therapy, manifested primarily by the onset of gradual increases in serum Cr and decreases in serum phosphorus [9]. However, no laboratory abnormalities of Cr or phosphorus were found in this short-term regimen study (10 mg daily for 7 days).

In clinical study, increases in ALT concentrations were the most frequent laboratory abnormalities at a 10-mg dose of ADV in patients with chronic hepatitis B and large increases in serum ALT concentrations associated with drug-related hepatic toxicity generally occurred with concurrent changes in bilirubin, ALB and PT [5].

Table 3Main pharmacokinetic parameters of PMEA in fasting and dining conditions ($n = 10$)

Parameter/summary statistic	Fasting group	Dining group	P-value
$AUC_{0-24\text{ h}}$ ($\mu\text{g l}^{-1}\text{ h}$)			
Geometric mean (95% CI) CV (%)	221 (200, 248) 17	196 (181, 227) 18	0.1621*
$AUC_{0-\infty}$ ($\mu\text{g l}^{-1}\text{ h}$)			
Geometric mean (95% CI) CV (%)	225 (204, 252) 17	200 (184, 232) 18	0.1246*
C_{max} ($\mu\text{g l}^{-1}$)			
Geometric mean (95% CI) CV (%)	24.8 (22.6, 27.6) 16	22.9 (20.4, 26.6) 22	0.3215*
V_d/F (ml kg^{-1})			
Arithmetic mean (95% CI) CV (%)	8.2 (7.2, 9.2) 18	4.7 (3.1, 6.3) 53	0.0166
CL/F ($\text{ml h}^{-1}\text{ kg}^{-1}$)			
Arithmetic mean (95% CI) CV (%)	0.60 (0.53, 0.67) 18	0.65 (0.48, 0.82) 40	0.4426
MRT (h)			
Arithmetic mean (95% CI) CV (%)	7.9 (7.6, 8.2) 5	8.2 (7.9, 8.5) 5	0.9356
$t_{1/2}$ (h)			
Arithmetic mean (95% CI) CV (%)	7.4 (6.8, 8.0) 12	7.0 (6.6, 7.4) 8	0.3201
t_{max} (h)			
Median (range)	1.00 (0.75–1.50)	2.75 (2.00–3.00)	< 0.0001

Statistical analysis was performed by ANOVA.*Following logarithmic transformation.

Figure 4

Mean PMEA concentration–time curves in fasting (◆) and dining (■) conditions ($n = 10$)

Figure 5

Mean concentration–time curves of PMEA on day 1 (◆) and day 7 (■) ($n = 10$)

In this study, increases in ALT concentrations were observed in both the single- and multiple-dose study and may have been caused by individual differences, since no group differences were found by ANOVA. It is known that ALT elevations are associated with a specific T-cell reaction in hepatitis B core antigen [10]. Increases in TBIL and IBIL concentrations were observed in the single-dose study, with significant differences from baselines by t -test. PT prolongations were observed in

both the single- and multiple-dose studies, but none was beyond the normal range. Much attention should be paid to CK and LDH elevations observed in this study.

ADV can be rapidly converted to PMEA following an oral dose and no intact prodrug or monoester has been detected in blood [11]. In this study, no ADV or monoester but PMEA was detected in human serum, in agreement with previous reports.

Table 4Main pharmacokinetic parameters of PMEA following multiple-dose of ADV ($n = 10$)

Parameter/summary statistic	Day 1	Day 7	P-value
AUC _{0-24 h} ($\mu\text{g l}^{-1} \text{h}$)			
Geometric mean (95% CI) CV (%)	238 (218, 258) 14	218 (193, 245) 19	0.6412*
AUC _{0-∞} ($\mu\text{g l}^{-1} \text{h}$)			
Geometric mean (95% CI) CV (%)	241 (220, 261) 14	221 (196, 248) 19	0.6330*
C_{max} ($\mu\text{g l}^{-1}$)			
Geometric mean (95% CI) CV (%)	24.8 (22.5, 27.5) 16	23.9 (20.9, 27.1) 21	0.7304*
V_d/F (ml kg^{-1})			
Arithmetic mean (95% CI) CV (%)	5.8 (5.1, 6.5) 19	5.7 (5.0, 6.4) 21	0.0811
CL/F ($\text{ml h}^{-1} \text{kg}^{-1}$)			
Arithmetic mean (95% CI) CV (%)	0.65 (0.59, 0.71) 15	0.67 (0.61, 0.72) 13	0.3434
MRT (h)			
Arithmetic mean (95% CI) CV (%)	7.7 (7.3, 8.1) 5	7.6 (7.4, 7.8) 5	0.1147
$t_{1/2}$ (h)			
Arithmetic mean (95% CI) CV (%)	6.5 (6.1, 6.9) 9	6.4 (5.9, 6.9) 9	0.4777
t_{max} (h)			
Median (range)	1.50 (0.75–2.50)	1.50 (0.75–2.00)	0.3353
C_{min} ($\mu\text{g l}^{-1}$)			
Geometric mean (95% CI) CV (%)	2.22 (1.83, 2.78) 25	2.32 (1.91, 2.72) 26	0.4532
C_{ss} ($\mu\text{g l}^{-1}$)			
Geometric mean (95% CI) CV (%)		10.3 (8.9, 11.7) 22	
DF (%)			
Geometric mean (95% CI) CV (%)		208 (191, 225) 13	

Statistical analysis was performed by paired *t*-test. *Following logarithmic transformation.

The pharmacokinetic parameters $t_{1/2}$, t_{max} and AUC following a single dose of ADV were similar to the reported data [5], but C_{max} was higher, which might be caused by physical differences. The mean C_{max} increased linearly with dose within the examined dose range, but AUC did not. Previous studies with adults have indicated dose-proportional increases in AUC [12, 13], although nonlinearities of C_{max} and AUC have been found in infants and children infected with HIV [11]. When a compound exhibits nonlinear pharmacokinetics, the AUC usually increases in a manner disproportionate to the applied dose. When the AUC increase is less than that expected on the basis of a linear relationship, it is indicative of either increased elimination, usually due to induction of metabolizing enzymes, or reduced absorption [14]. However, PMEA is not metabolized by P450 [2], the absorption of PMEA is not saturated at doses of 60–500 mg [11] and the V_d/F , CL/F, MRT, $t_{1/2}$ and urinary excretion rates of PMEA were similar ($P > 0.05$) at the three dose levels, so it is difficult to explain the nonlinearity of AUC. Larger numbers of subjects and more dose levels will need to be studied for an explanation.

The urinary excretion of PMEA could be saturated at high dose and its urinary recovery rate descended when saturated. The urinary recovery of PMEA was 44.4 ± 15.6 , 32.8 ± 6.06 and $29.6 \pm 12.2\%$, respectively, following a single dose of ADV 125, 250 and 500 mg [15], but 34.3 (31.4, 36.6), 36.2 (29.5, 42.5) and 35.0 (25.8, 44.1)% in this study, respectively, following a single dose of ADV 20, 40 and 40 mg, indicating that urinary excretion of PMEA was not saturated after an oral dose of ADV up to 40 mg. The mean oral bioavailability of PMEA exceeded 35% from different doses of ADV on the basis of the urinary recovery of PMEA 24 h postdose, which was similar to another report [5].

Compared with those in the fasting group, prolonged ($P < 0.01$) t_{max} was achieved in nonfasting group, but AUC, $t_{1/2}$, MRT and C_{max} of these two groups were similar ($P > 0.05$), indicating that food did not affect the extent but the rate of the absorption of PMEA after oral dosing.

In the multiple-dose study, the steady-state concentration (C_{ss}) was achieved on day 4 and the pharmacokinetic parameters of PMEA were similar to those

following a single dose of ADV, without accumulation following 7 days of once-daily dosing of ADV 10 mg. A higher DF% of PMEAs in serum was achieved because the ADV tablet was not a sustained-release but an ordinary preparation, and a prolonged intracellular half-life (12–36 h) of the active antiviral intracellular anabolite (PMEA diphosphate) accounted for the once-daily dosing.

PMEA acts in cells, but the relationship between the concentrations of PMEAs in cells and in serum is not known and further research is needed.

In conclusion, ADV is safe and well tolerated in healthy Chinese subjects at a single dose of 5–60 mg or 10 mg once daily for 7 days. No serious adverse events occurred during the study and all subjects were in good compliance. The mean $C_{\max}$ of PMEAs is proportional to dose following a single oral dose of ADV 10, 20 and 40 mg, but the linearity of AUC needs further study. The steady-state pharmacokinetic parameters of PMEAs are similar to those following a single dose, without accumulation following 7 days of once-daily dosing of ADV 10 mg. The AUC of PMEAs is not affected by food.

References

- Buti M, Esteban R. Adefovir dipivoxil. *Drugs Today (Barc)* 2003; 39: 127–35.
- Shaw JP, Louie MS, Krishnamurthy VV, Arimilli MN, Jones RJ, Bidgood AM, Lee WA, Cundy KC. Pharmacokinetics and metabolism of selected prodrugs of PMEAs in rats. *Drug Metab Dispos* 1997; 25: 362–6.
- Ray AS, Vela JE, Olson L, Fridland A. Effective metabolism and long intracellular half life of the anti-hepatitis B agent adefovir in hepatic cells. *Biochem Pharmacol* 2004; 68: 1825–31.
- Seigneres B, Aguesse-Germon S, Pichoud C, Vuillermoz I, Jamard C, Trepo C, Zoulim F. Duck hepatitis B virus polymerase gene mutants associated with resistance to lamivudine have a decreased replication capacity in vitro and in vivo. *J Hepatol* 2001; 34: 114–22.
- FDA. Advisory Committee Briefing Document. Adefovir Dipivoxil for the Treatment of Chronic Hepatitis B. Available at http://www.fda.gov/ohrms/dockets/ac/02/briefing/3885B1-01_Gilead.pdf (last accessed: 17 October 2005).
- Cundy KC, Barditch-Crovo P, Walker RE, Collier AC, Ebeling D, Toole J, Jaffe HS. Clinical pharmacokinetics of adefovir in human immunodeficiency virus type 1-infected patients. *Antimicrob Agents Chemother* 1995; 39: 2401–5.
- Cundy KC. Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir. *Clin Pharmacokinet* 1999; 36: 127–43.
- Ji-dong JIA, Zhuang H. The overview of the seminar on chronic hepatitis B. *Chin J Hepatol* 2004; 12: 698–9.
- Dusheiko G. Adefovir dipivoxil for the treatment of HBeAg-positive chronic hepatitis B: a review of the major clinical studies. *J Hepatol* 2003; 39 (Suppl. 1): S116–23.
- Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, Gutfreund K, Lamy P, Murray A. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *Hepatology* 2000; 32: 129–34.
- Hughes WT, Shenep JL, Rodman JH, Fridland A, Willoughby R, Blanchard S, Purdue L, Coakley DF, Cundy KC, Culnane M, Zimmer B, Burchett S, Read JS. Single-dose pharmacokinetics and safety of the oral antiviral compound adefovir dipivoxil in children infected with human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 2000; 44: 1041–6.
- Barditch-Crovo P, Toole J, Hendrix CW, Cundy KC, Ebeling D, Jaffe HS, Lietman PS. Anti-human immunodeficiency virus (HIV) activity, safety, and pharmacokinetics of adefovir dipivoxil (9-[2-(bis-pivaloyloxymethyl)-phosphonylmethoxyethyl]adenine) in HIV-infected patients. *J Infect Dis* 1997; 176: 406–13.
- Deeks SG, Collier A, Lalezari J, Pavia A, Rodrigue D, Drew WL, Toole J, Jaffe HS, Mulato AS, Lamy PD, Li W, Cherrington JM, Hellmann N, Kahn J. The safety and efficacy of adefovir dipivoxil, a novel anti-human immunodeficiency virus (HIV) therapy, in HIV-infected adults: a randomized, double-blind, placebo-controlled trial. *J Infect Dis* 1997; 176: 1517–23.
- Setchell KD, Brown NM, Desai PB, Zimmer-Nechimias L, Wolfe B, Jakate AS, Creutzinger V, Heubi JE. Bioavailability, disposition, and dose–response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. *J Nutr* 2003; 133: 1027–35.
- Gilead Sciences. A Phase I/II Study of the Safety, Tolerance, Pharmacokinetics and Anti-HIV Activity of 9-[2-(Bis-pivaloyloxymethyl) Phosphonomethoxy ethyl]Adenine (bis-POM PMEAs) and Placebo in HIV-Infected Patients. GS-94-402. : Gilead Sciences Protocol Inc., Foster City, CA. 1994.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 Adverse events related to ADV

Table S2 Laboratory abnormalities related to ADV in single-dose trial

Table S3 Laboratory abnormalities related to ADV in multiple-dose trial

Table S4 Thrombotest and blood biochemical profiles of the subjects (mean $\pm$ SD)

Table S5 Cumulative urinary excretion (mg) of PMEAs following single-dose of ADV n=9, arithmetic mean (95%CI) CV (%)

Table S6 Main pharmacokinetic parameters of PMEAs in male and female subjects n=10

Figure S1 Mean concentration-time curves of PMEAs in male and female subjects (n=10)

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