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Pooled model-based approach to compare the pharmacokinetics of entecavir between Japanese and non-Japanese chronic hepatitis B patients

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Abstract

This study evaluated the population pharmacokinetics (PK) of entecavir in Japanese patients with chronic hepatitis B infection enrolled in 2 Japanese phase IIb clinical trials and compared them to non-Japanese patients enrolled in global phase II trials. The objectives were to identify significant and clinically meaningful covariate effects on entecavir population pharmacokinetic parameters and assess whether differences exist between Japanese and non-Japanese patients. A total of 843 observations were obtained from 142 patients who received once daily administration of entecavir at 0.1, 0.5, and 1.0 mg doses in the 2 Japanese studies. Consistent with findings in non-Japanese patients, creatinine clearance estimated with ideal body weight (ICrCL) was found to be statistically significant for clearance in a 2-compartment model. Also, the entecavir dose was identified as a covariate on intercompartmental clearance. Age, gender, and hepatic function were not identified as covariate for clearance. The estimated population average of oral clearance in a typical patient with a reference ICrCL value of 100 mL/min was 26.4 L/h (interindividual variability: 19.4%). This model-based analysis indicates that the PK of entecavir are similar in Japanese and non-Japanese chronic hepatitis B patients.

Keywords: Entecavir; Pharmacokinetics; Chronic hepatitis B; Japanese

1. Introduction

Entecavir (ETV), a cyclopentyl guanosine analog, is a potent and selective inhibitor of hepatitis B virus (HBV) DNA polymerase that inhibits all 3 activities of the HBV polymerase: base priming, reverse transcription of the negative strand from pre-genomic messenger RNA, and synthesis of the positive strand of HBV DNA (Seifer et al., 1998). The metabolism of ETV was evaluated in vitro and in vivo; it was found to be a substrate, inhibitor, nor inducer of the cytochrome P450 enzyme system. It is predominantly eliminated by the kidneys, with urinary recovery of unchanged drug at steady state ranging from 78% to 80% of the administered dose and a mean renal clearance of 372.1 and 366.4 mL/min at ETV doses of 0.5 and 1 mg, respectively, in Japanese healthy subjects (Amano and Goto, 2007; Ishikawa, 2006). Because the renal clearance exceeded glomerular filtration rate, tubular secretion is likely to play a role in its elimination. In subjects with renal impairment, there is a strong correlation between decreased creatinine clearance (CrCL) and increased exposure to ETV (Matthews, 2006).

In phase I studies, the maximum plasma concentration (C_{max}) of ETV and the area under the concentration–time curve (AUC) at steady state (AUC_{ss}) increased in proportion to dose and were similar among healthy subjects in the United States, Japan, and China (Matthews, 2006; Yan et al., 2002). Following multiple oral doses of ETV 0.5 mg in healthy subjects in the United States (n = 6), Japan (n = 6), and China (n = 8), mean (SD) AUCs were 15.0 (2.8), 17.8 (1.3), and 17.4 (2.8) ng·h/mL, respectively. A food effect was observed on ETV pharmacokinetics (PK): the C_{max} was decreased by 44–46%, and the AUC was decreased by 18–20% when ETV was taken with food

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(Matthews, 2006). Therefore, ETV was administered at fasting state in clinical phase II and III studies.

A population PK analysis was conducted to characterize the PK of ETV in chronic hepatitis B (CHB) patients using data obtained from 3 phase II studies in non-Japanese patients (Zhu et al., 2008). A 2-compartment model was used to estimate individual PK parameters, and apparent clearance (CL/F) was found to increase with increasing calculated CrCL based on ideal body weight (ICrCL). The CL/F of a typical patient with a reference ICrCL value of 100 mL/min was 27.1 L/h, and the apparent volumes of distribution of the central and peripheral compartments were 115 and 1830 L, respectively.

The ETV development program in Japan compared the PK profiles of Japanese and non-Japanese patients in phase II clinical studies. If these were found to be comparable, then the response to ETV therapy in Japanese patients was bridged to the worldwide database of non-Japanese CHB patients. To accomplish this, we characterized the population PK of ETV in Japanese CHB patients using data from 2 Japanese phase II clinical trials (studies AI463052 and AI463053), which was not used for the previous population PK analysis, and identified covariate effects on ETV PK parameters of these patients. We also performed a comparative PK analysis by a pooled model-based covariate method, using integrated concentration data to assess whether the PK of ETV in Japanese CHB patients differed from that of the non-Japanese CHB patients in the global studies.

2. Materials and methods

2.1. Data

The Japanese ETV population PK model was developed with 843 ETV plasma concentration observations from 142 Japanese CHB patients. These patients were enrolled in 2 phase II clinical studies (studies AI463052 and AI463053) that were conducted to evaluate the efficacy, safety, and PK of ETV. Table 1 presents a summary of the baseline demographic and laboratory data from the Japanese CHB patients who were included in the population PK analysis (77 patients from study AI463052 and 65 patients from study AI463053). No difference in demographic and laboratory data was observed between the 2 studies.

Study AI463052 was a randomized, double-blind, parallel-arm study with 2 ETV dose arms (0.5 and 1.0 mg, each taken once daily for 52 weeks) in Japanese CHB patients who were refractory to lamivudine therapy (Amano and Goto, 2007; Ishikawa, 2006; Suzuki et al., 2008). Study AI463053 was a randomized, double-blind, parallel-arm study with 2 ETV dose arms (0.1 and 0.5 mg, each taken once daily for 52 weeks) in nucleoside-naive Japanese CHB patients (Amano and Goto, 2007; Ishikawa, 2006; Kobashi et al., 2009). The study protocols and patient informed consents were approved by an institutional review board at each study center and conducted in accordance with the principles of the declaration of Helsinki and Good Clinical Practice.

Table 1
Summary of baseline demographics for pharmacokinetic database in the Japanese clinical trials

Baseline characteristic (units)	Mean (SD)	Median	Range
Age (y)	44 (10)	43	24–68
Weight (kg)	66.5 (13.1)	65.0	39.0–118.2
Body mass index (kg/m^2)	23.7 (3.8)	23.6	17.3–42.9
ICrCL[a] (mL/min)	101.9 (21.1)	102.3	54.2–150.0
Total bilirubin (mg/dL)	0.6 (0.3)	0.6	0.2–2.2
Alkaline phosphatase (IU/L)	312 (155)	273.5	127–1280
Albumin (g/dL)	4.4 (0.4)	4.4	3.1–5.2
Alanine aminotransferase (IU/L)	143 (137)	107	24–1250
Aspartate aminotransferase (IU/L)	88 (75)	69.5	21–634
Amylase (IU/L)	142 (42)	133.5	43–298
Gender, *n* (%)	Male: 116 (82), female: 26 (18)		
Study, *n* (%)	AI463052: 77 (54), AI463053: 65 (46)		
Dose, *n* (%)	0.1 mg: 32 (23%), 0.5 mg: 72 (51%), 1.0 mg: 38 (27%)		

[a] ICrCL, creatinine clearance calculated by Cockcroft and Gault equation using ideal body weight (Cockcroft and Gault, 1976; Devine, 1974).

Plasma samples for measurement of ETV concentration in both studies were collected at the following times: predose (trough); 1.5 ± 0.25, 3 ± 0.25, and 10 ± 0.25 h post-dose at the week 2, 4, or 48 visits. This sampling design, which was close to the scheme developed by D-optimal design (trough, 0.2, 1.4, 3.5, and 10 h post-dose) (Zhu et al., 2008), was selected for accurate and precise estimation of CL/F, a key determinant of ETV exposure. All sampling collections were conducted for ETV concentrations at steady state that would provide the decisive information for accurate and precise estimation of CL/F (Ogata, 2007; Tsuchiwata et al., 2005). Sampling points were identified at 1.5 and 3.0 h post-dose from the alpha phase assumed in the 2-compartment model, and 10 h post-dose and trough level from the beta phase based on the plasma concentration–time profiles in the Japanese phase I study (data not shown). Sparse samples were also collected randomly at any time after 1 h post-dose at the week 12, 24, and 36 visits.

The comparative PK analysis was performed by pooling the data from the Japanese phase II studies (described above) with data from 3 global phase II clinical trials (studies AI463004, AI463005, and AI463014) in CHB patients, which have been used to develop the population PK model in non-Japanese patients (LaCreta et al., 2005; Zhu et al., 2008). A total of 1000 ETV plasma concentration measurements from 177 non-Japanese patients were available in the population PK analysis data set.

Study AI463004 was a randomized, double-blind, placebo-controlled, dose-escalation study with 4 ETV dose levels (0.05, 0.1, 0.5, and 1.0 mg, each taken once daily for 28 days), in non-Japanese CHB patients who were either treatment naive or lamivudine/interferon pretreated (De Man et al., 2001). ETV PK samples were collected before the ETV dose on days 1, 7, 14, 21, and 28 of study AI463004,

and data were available for 30 patients. Study AI463005 was a randomized, double-blind, parallel-arm study with 3 ETV dose levels (0.01, 0.1, and 0.5 mg, each taken once daily for 24 weeks) in non-Japanese CHB patients (Lai et al., 2002). In this study, ETV PK data were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 or 24 h post-dose on day 1 and on weeks 4, 12, and 22; data were available for 120 patients. Study AI463014 was a randomized, double-blind study with 3 ETV dose levels (0.1, 0.5, and 1.0 mg, each taken once daily for 48 weeks) (Chang et al., 2005). ETV PK samples were collected 1.5, 3, and 6 h post-dose on day 1 and on weeks 4, 12, 24, 36, and 48 at least 1 h post-dose, and PK data were available for 72 non-Japanese patients.

2.2. ETV plasma concentration assay

ETV plasma concentrations were determined by a validated liquid chromatography tandem mass spectrometry assay with a lower limit of quantification of 0.005 ng/mL (Yan et al., 2002). The assay was sensitive and specific, and was based on solid-phase extraction of ETV from plasma samples before chromatographic separation and selected reaction-monitoring detection by mass spectrometry. A structural analog, lobucavir, was used as the internal standard. Separation of analyte and internal standard from the matrix was achieved using reversed-phase liquid chromatography with gradient elution. Eluted analyte and internal standard were monitored by specific ion transitions using tandem mass spectrometric detection. Plasma samples from Japanese CHB patients in studies AI463052 and AI463053 were analyzed for ETV in a total of 18 analytical runs. The standard curve, which ranged from 0.005 to 25 ng/mL for ETV, was fitted using a $1/x$ weighted quadratic regression model. The between-run and within-run precision values for analytical quality control samples were no greater than 5.6% and 18.8% coefficient of variation, respectively, with deviations from the nominal concentrations of no more than ±7.7%. Because all samples were collected at steady state, only one sample showed the lower limit of quantification and was treated as missing data.

2.3. Population PK model development

Plasma concentration–time data from Japanese CHB patients in studies AI463052 and AI463053 were analyzed using a nonlinear mixed-effects modeling approach, as implemented in the NONMEM computer program (version V, level 1.1, GloboMax®). The plasma concentration–time data were fitted to a 2-compartment model with first-order absorption and elimination, using the first-order conditional estimation method with interaction to estimate the population and individual PK parameters. Interindividual variability was estimated by an exponential model, and an additive and proportional error model was employed for the estimation of the random residual variability. The result of the Japanese phase I study, in which an intensive sampling design was employed for noncompartmental analysis, suggested the difficulty of precise estimation of the absorption rate constant (KA) and its variability (Amano and Goto, 2007; Ishikawa, 2006) since ETV was rapidly absorbed with a median time to maximum plasma concentration (T_{max}) of 0.6–0.75 h at steady state. Therefore, the sensitivity test was conducted on fixed KA values ranging from 0.5 to 6.0 h^{-1}.

Covariate selection was performed by forward inclusion and backward elimination based on the likelihood ratio test. Covariate–parameter relationships significant at a 5% level were included at forward inclusion steps, and were retained in the model at backward elimination steps, provided they were significant at a 0.1% level (Jonsson and Karlsson, 1998).

The following continuous and categorical covariates were investigated: body weight, ideal body weight (IBW) (Winter, 2003), age, body mass index, ICrCL (Cockcroft and Gault, 1976), total bilirubin, alkaline phosphatase, albumin, alanine aminotransferase, aspartate aminotransferase, amylase, sex, and drug dosage. IBW was calculated as described in Equations 1 and 2:

$$\text{Males}: \ \text{IBW[kg]} = 50 + 0.906 \times (\text{Height[cm]} - 152.4) \quad (1)$$

$$\text{Females}: \ \text{IBW[kg]} = 45 + 0.906 \times (\text{Height[cm]} - 152.4) \quad (2)$$

ICrCL was calculated as described in Equations 3 and 4:

$$\text{Males}: \ \text{ICrCL[mL/min]} = (140 - \text{Age[yrs]}) \times \text{IBW[kg]} / (72 \times \text{SCr[mg/dL]}) \quad (3)$$

$$\text{Females}: \ \text{ICrCL[mL/min]} = \text{Male ICrCL[mL/min]} \times 0.85 \quad (4)$$

SCr represents serum creatinine value. Covariate–parameter relationships for continuous value covariates were described by power covariate models, and the relationships for categorical covariates were described by proportional shift covariate models.

Other model assessment criteria for goodness of fit included checking for reductions in interindividual variability and random residual variability, visual improvements in agreement between the observed and predicted plasma concentrations, and the reduction of nonconditional and no eta–epsilon interaction weighted residuals for the predicted concentrations.

2.4. Population PK model evaluation

The stability of covariate effects included in the final model was evaluated by nonparametric bootstrap (Ette, 1997; Parke et al., 1999) and permutation analyses (Good, 2000), and the predictive performance of the final model was evaluated by a predictive check.

Briefly, the nonparametric bootstrap analysis was performed by fitting the final and reduced (excluding a final model covariate effect) models to 200 bootstrapped data sets randomly generated without any stratification, and determining the percentage of bootstrapped data sets for which covariate effect was significant at a 1% level. The permutation test was performed by fitting the final model to 1000 data sets in which the association between patients and covariate values had been randomly scrambled (permuted) without correlation between covariates (the ETV dose and ICrCL). The likelihood that the effect of a covariate was included by random chance in the final model was evaluated by determining the percentage of these fits that had a NONMEM objective function value (equivalent to −2 log likelihood) that was lower than that of the final model fit. The predictive check was performed by comparing statistics of observed concentration data with the distributions of the corresponding statistics obtained from the final model by Monte Carlo simulation. Model-estimated CL/F and AUC_{ss} were also compared to values obtained by noncompartmental analysis.

2.5. Comparison of population PK between Japanese and non-Japanese populations

ETV exposure measures (steady-state C_{max} and AUC_{ss}) of Japanese patients were calculated using empirical Bayesian estimates determined in the Japanese population PK analysis. AUC_{ss} was calculated by dividing the ETV dose by CL/F. C_{max} was the highest simulated concentration obtained from steady-state simulation by 0.01 h after dosing. Distributions of ETV exposure measures in Japanese patients were compared with those of non-Japanese patients derived from non-Japanese population PK analysis (Zhu et al., 2008); results were described by dose and population box plots.

Furthermore, the potential effect of Japanese ethnicity on ETV CL/F was assessed by combining the Japanese and non-Japanese population PK analysis data sets and by performing a likelihood ratio test to test for this covariate effect added to the final model.

The sampling design employed for accurate and precise estimation of CL/F in Japanese phase II studies (studies AI463052 and AI463053) did not include sampling points after the first dose (required for estimation of distribution volume at steady state [Vdss] (Ogata, 2007; Tsuchiwata et al., 2005). Therefore, the potential ethnic effect was tested only for CL/F and not distribution Vd_{ss}.

3. Results

3.1. Population PK model development

The ETV PK data in Japanese CHB patients were described by a 2-compartment population PK model, identical in structure to the model used to describe the PK of ETV in non-Japanese CHB patients (LaCreta et al., 2005; Zhu et al., 2008). The model was parameterized in terms of CL/F, the apparent volumes of distribution of the central and peripheral compartments, intercompartmental clearance (Q/F), and KA, and included covariate effects of ICrCL on CL/F and ETV dose on Q/F.

The Japanese population PK model parameter estimates are presented in Table 2. The relative standard error of the parameters specifying CL/F was reasonably small, indicating that CL/F, a key determinant of ETV exposure, was adequately estimated in the final population PK model. Also, the effect of the study (the ratio of study AI463053 to study AI463052) on CL/F was 1.06 and its asymptotic-associated 95% confidence interval was 0.98–1.15, indicating that it was reasonable to use the integrated Japanese data for the population PK analysis.

As in the case of the population PK analysis in non-Japanese patients, KA could not be reliably estimated because of the sampling design and was fixed to a value of 3.5 h^{-1} based on the sensitivity analysis. ETV T_{max} derived from the KA value was 0.6 h for Japanese patients. This was similar to the value obtained in a population PK analysis of non-Japanese patients (~0.5 h) (Zhu et al., 2008), and median values at steady state (0.6–0.75 h) in a Japanese phase I study, in which an intensive sampling design was employed

Table 2
Summary of parameter estimates for the final pharmacokinetic model

Parameter[a]		Population mean (95% CI[a])	Percentage CV[b] interindividual variance (% SEM)
CL/F (L/h)	θ1	16.0 (11.2 to 20.8)	19.4 (24.0)
Effect of ICrCL	θ2	10.4 (5.8 to 15.0)	
Q/F (L/h)	θ3	913 (−37 to 1863)	43.6 (39.8)
Effect of Dose	θ4	−0.532 (−0.704 to −0.360)	
V2/F (L)	θ5	114 (75 to 153)	26.0 (50.1)
V3/F (L)	θ6	642 (257 to 1027)	53.9 (87.8)
KA (h^{-1})	θ7	3.50 (fixed)	Not estimated
CCV[c] residual error (as % CV)			30.7 (12.4)

Additive residual error was <0.0001 ng/mL. CI = confidence interval; CCV= constant coefficient of variation.

[a] Asymptotic–associated 95 % confidence intervals.

[b] Percentage SEM = standard error/parameter estimate × 100.

for noncompartmental analysis (Amano and Goto, 2007; Ishikawa, 2006). One major limitation of this study was that the first observation at steady state was taken at approximately 2- to 3-fold T_{max} derived from the fixed KA (~1.5 h). However, almost all individual T_{max} values at steady state were within 1 h post-dose in the Japanese phase I study (unpublished data), suggesting that the impact of fixed KA value is limited on the PK.

The covariate–parameter relationships between CL/F and ICrCL, and between Q/F and ETV dose, were specified according to Equations 7 and 8, respectively:

$$CL/F = \theta 1 + \theta 2 \times (ICrCL/100) \quad (7)$$

$$Q/F = \theta 3 * DOSE^{\theta 4} \quad (8)$$

The concentration–time profiles and diagnostic plots shown in Figs. 1 and 2 illustrate the good agreement between observed and model-predicted ETV concentrations and the lack of apparent systematic bias dependent on time and concentrations. Fig. 3 illustrates the covariate–parameter relationships included in the final population PK model.

3.2. Population PK model evaluation

The stability of covariate effects included in the final population PK model was confirmed by nonparametric bootstrap and permutation test analysis. The covariate effects of ICrCL were statistically significant in 82% and 87% of the bootstrap likelihood ratio test analyses, respectively. Furthermore, the NONMEM objective function values of the final population PK model fitted to the population PK analysis data set were lower than the objective function values obtained when this model was fitted to the data sets in which the association between patient and ICrCL or dose (as covariate) was permuted (1000 permuted data sets for each covariate). These results are consistent with the 0.1% level of significance employed in the backward elimination step of covariate selection.

A predictive check was conducted to evaluate the performance of the final model. Monte Carlo simulation was employed to generate 1000 trials with the final model. The 25th, 50th, and 75th percentiles of simulated concentrations were computed for trough and peak concentrations (i.e., pre-dose and nominal 1.5-h time points after administration of ETV 0.5 mg). The percentile statistics of the observed concentrations were within the central 99% of corresponding simulated distributions, with the exception of the 25th percentile of the 2-h time points (Fig. 4). Furthermore, the model-estimated distributions CL/F and AUC_{ss} (Table 3), which are determinants and measures of ETV exposure, were compared and found to be similar to those calculated by noncompartmental analysis in healthy subjects (Amano and Goto, 2007).

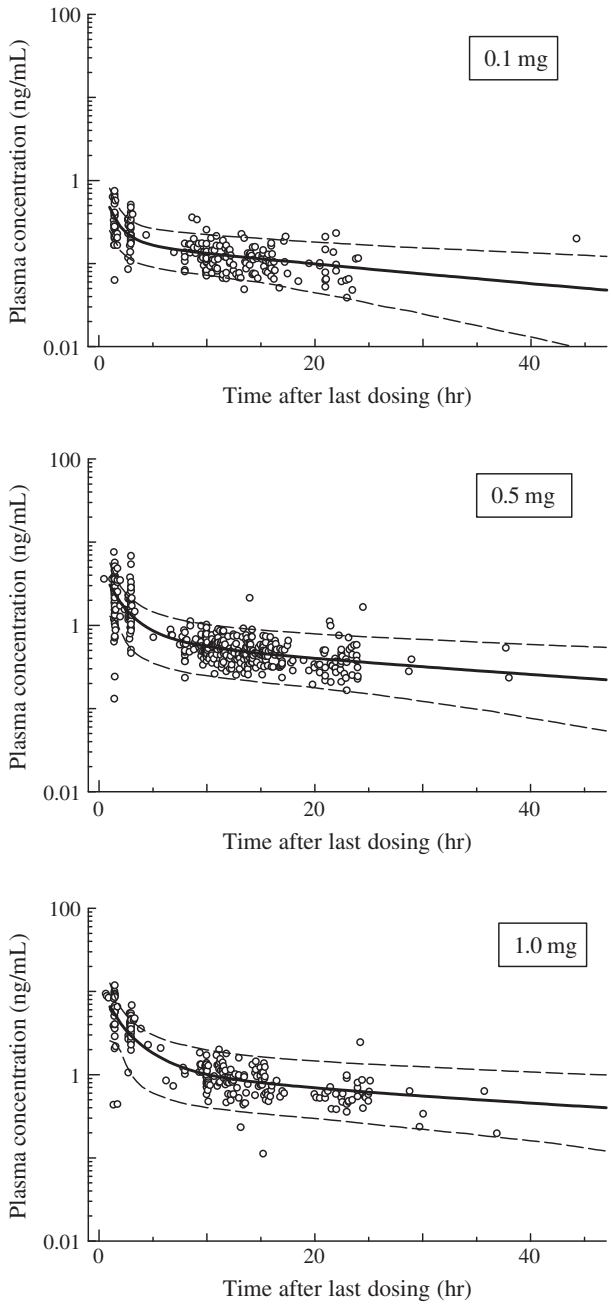

Fig. 1. Observed plasma concentrations versus 95% prediction intervals of simulated data at steady state. Solid line represents median of simulated data. Dashed lines show lower and upper limit of 95% prediction intervals.

3.3. Comparison of population PK between Japanese and non-Japanese populations

AUC_{ss} was calculated based on individual CL/F and C_{max} values obtained from steady-state simulation. AUC_{ss} was not supported by observations during the first 1.5 h post-dose because of the sampling design and mean (SD) of the fraction for AUC_{ss} during the first 1.5 h. This, expressed as AUC1.5hr/AUC24hr, was 23.4% (3.5%). However, as mentioned above, it was considered that the

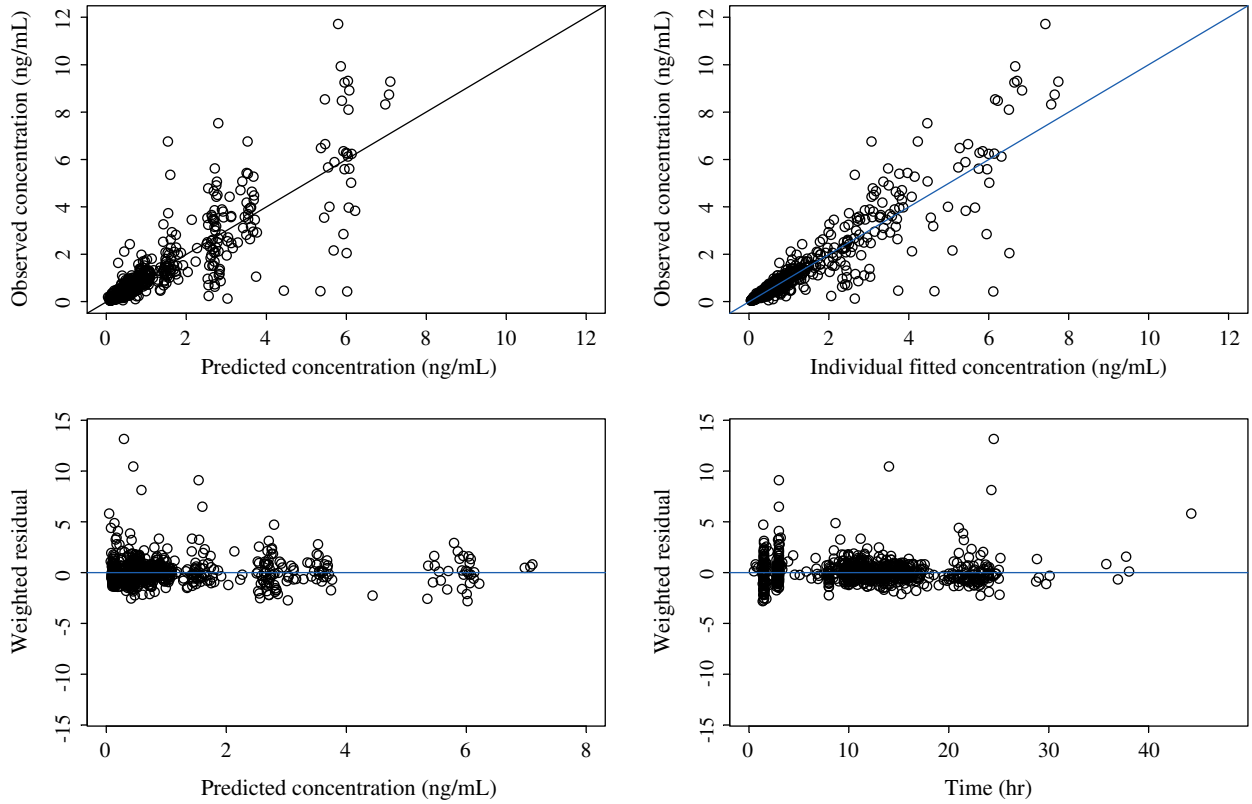

Fig. 2. Basic goodness of fit diagnostic plots for population PK model. Predicted concentration versus observed concentration, individual-fitted concentration versus observed concentration, nonconditional and no eta–epsilon interaction weighted residuals versus predicted concentration, and weighted residuals versus time.

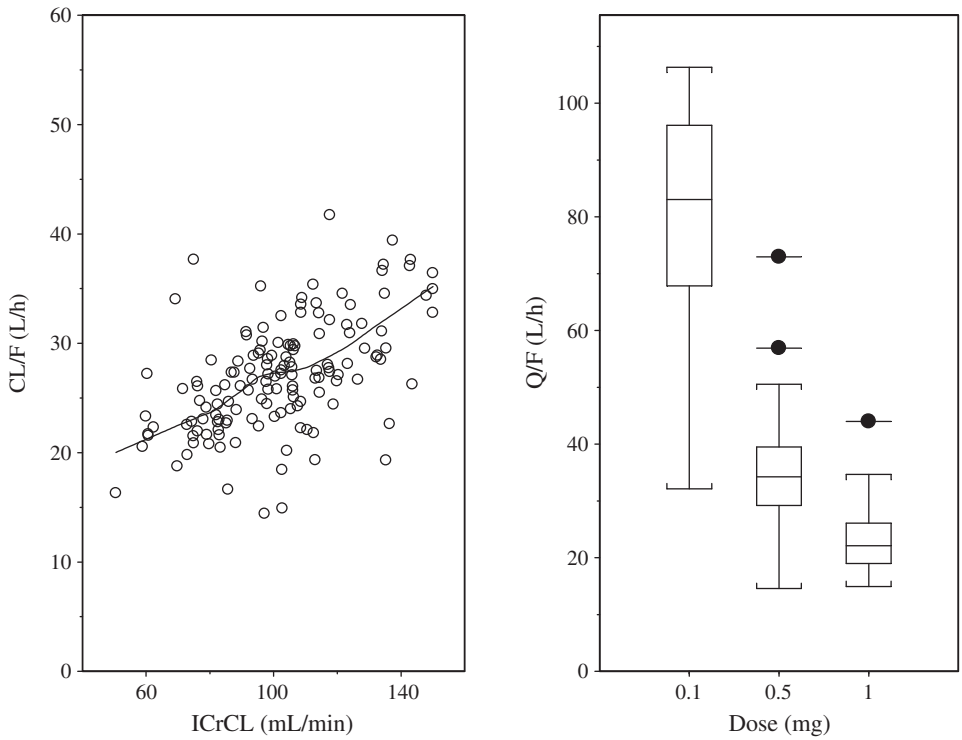

Fig. 3. Relationship between covariates and pharmacokinetic parameters. Creatinine clearance calculated using ideal body weight (ICrCL) versus apparent drug clearance (CL/F) overlaid with a smooth line, and box-and-whisker plot of Q/F for ETV doses.

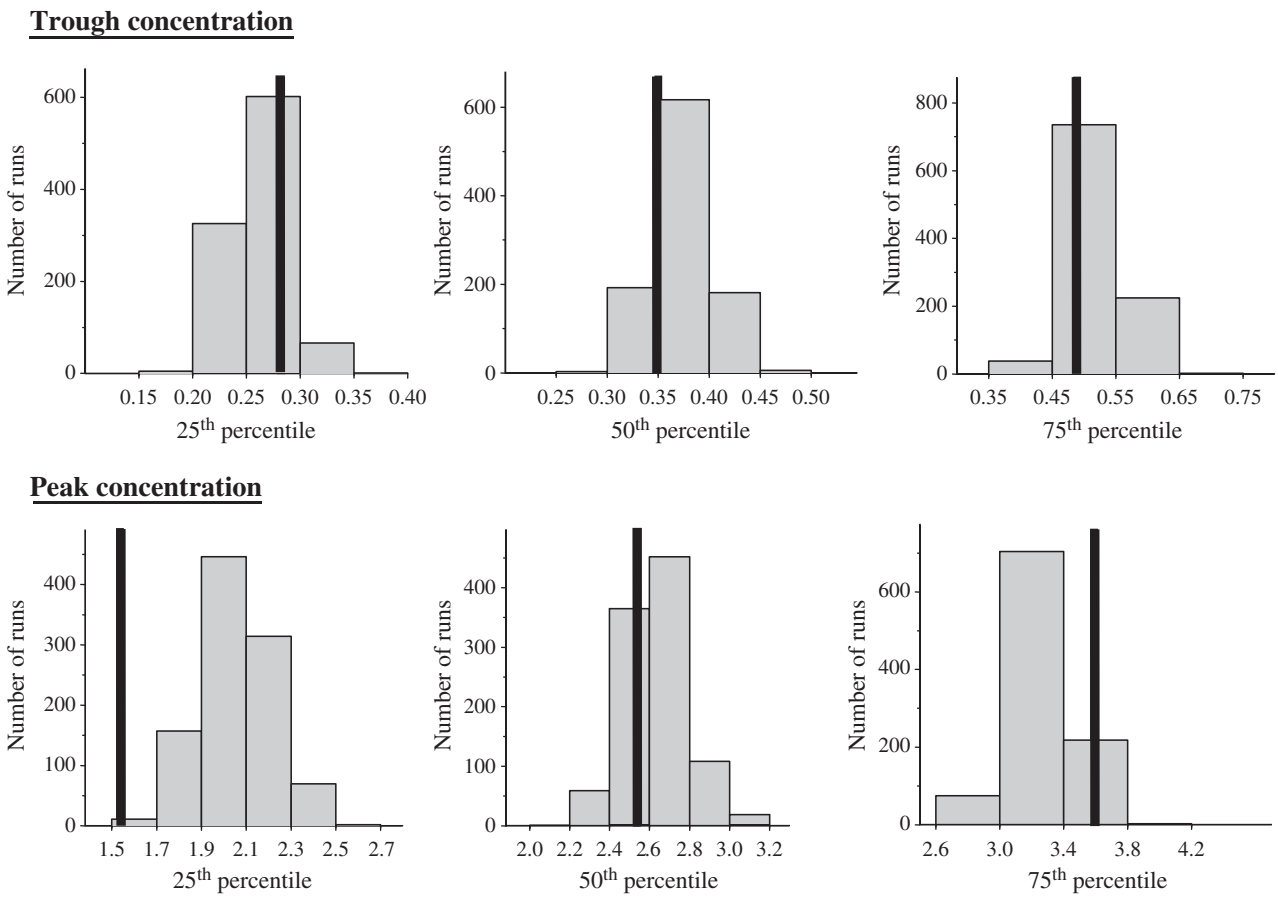

Fig. 4. Predictive check for 0.5 mg of ETV. The 25th, 50th, and 75th percentiles of simulated concentrations are shown for trough (C_{min}; upper panel) and peak (1.5 h post-dose; lower panel) concentrations. The vertical solid lines are drawn at corresponding statistics for observed data.

impact of fixing the KA value was limited in CL/F (i.e., AUC_{ss}) since ETV was rapidly absorbed, with the median T_{max} values at steady state (0.6–0.75 h) (Amano and Goto, 2007; Ishikawa, 2006) similar to the estimated T_{max}-derived fixed KA value (0.6 h), and almost all individual T_{max} values at steady state were within 1 h post-dose in the Japanese phase I study (unpublished data).

Table 3
Summary statistics for derived pharmacokinetic parameters at steady state

Parameter	Dose (mg)	Mean	SD
CL/F (mL/min)	0.1 (n = 32)	468.6	99.9
	0.5 (n = 72)	442.4	81.3
	1.0 (n = 38)	447.7	79.3
AUCss (ng·h/mL)	0.1 (n = 32)	3.72	0.82
	0.5 (n = 72)	19.56	4.14
	1.0 (n = 38)	38.34	6.55
C_{max} (ng/mL)	0.1 (n = 32)	0.632	0.046
	0.5 (n = 72)	3.601	0.458
	1.0 (n = 38)	7.259	0.636
C_{min} (ng/mL)	0.1 (n = 32)	0.087	0.028
	0.5 (n = 72)	0.382	0.123
	1.0 (n = 38)	0.653	0.212

Median values of non-Japanese and Japanese HBV patients at 0.1, 0.5, and 1.0 mg were 3.7, 18.7, and 46.9 ng·h/mL and 3.7, 18.4, and 38.2 ng·h/mL for AUC_{ss}, respectively, and 0.67, 3.9, and 8.9 ng/mL and 0.62, 3.5, and 7.1 ng/mL for C_{max}, respectively. Box plots of C_{max} and AUC_{ss} for ETV in Japanese and non-Japanese patients are given in Fig. 5. The distributions of AUC_{ss} at 0.1–1.0 mg for Japanese patients fell within the range of AUC_{ss} for non-Japanese CHB patients in studies AI463004, AI463005, and AI463014. However, the median AUC_{ss} of Japanese patients at 1.0 mg was lower than that of non-Japanese patients by approximately 20%. A similar trend was observed for C_{max}. The effect of Japanese ethnicity on ETV CL/F was further investigated by a pooled analysis of the Japanese and non-Japanese population PK analysis data sets. The effect of population (the ratio of Japanese to non-Japanese) on CL/F was tested by the likelihood ratio test and found to be insignificant as a covariate (P = 0.309). The mean (SE) ratio of this ethnicity covariate effect on CL/F was 0.962 (0.0408) and its asymptotic-associated 95% confidence interval was 0.882–1.042, indicating that the observed differences in ETV exposure are not due to an effect of ethnicity on ETV CL/F. The observed lower median ETV AUC_{ss} at 1.0 mg in

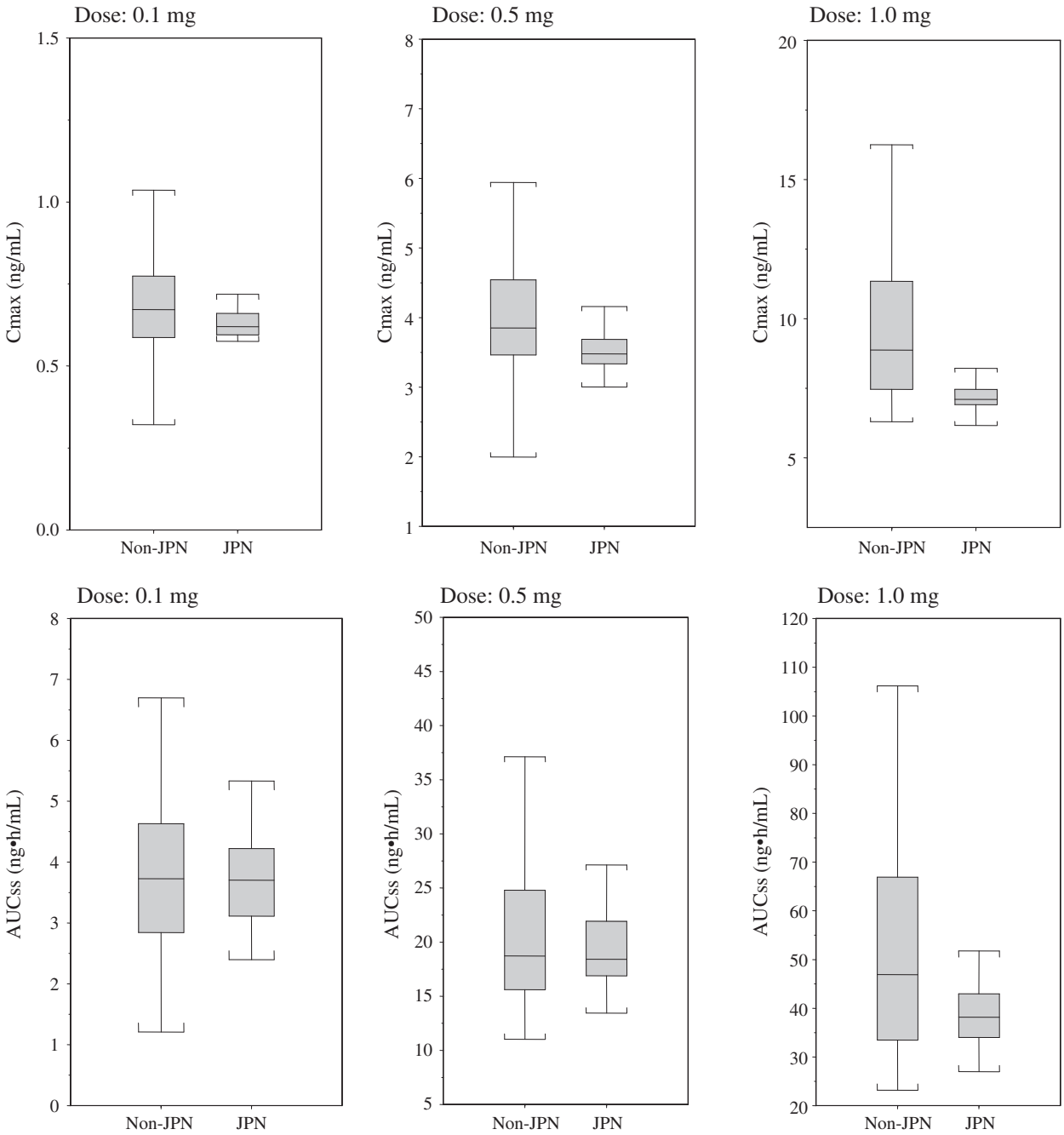

Fig. 5. Box plots of peak concentrations (C_{max}; upper panels) and area under the concentration–time curves at steady state (AUC_{ss}; lower panels) for 0.1, 0.5, and 1.0 mg of ETV in Japanese (JPN) and non-Japanese patients with HBV infection.

Japanese patients is consistent with the higher median ICrCL in Japanese patients (100.3 mL/min) compared with non-Japanese patients (81.5 mL/min).

Vdss, expressed as apparent volumes of distribution in the central compartment (V2/F) + apparent volumes of distribution in the peripheral compartment (V3/F), was approximately 3-fold larger in non-Japanese patients than in Japanese patients (1945 versus 756 L, respectively) (Zhu et al., 2008). Sampling collections after first dosing would provide the decisive information regarding Vdss (Ogata, 2007; Tsuchiwata et al., 2005). The sampling design employed in Japanese clinical phase II studies did not have sample collections after first dosing at all; however, the sampling design in clinical phase II studies in non-Japanese patients included blood collections at multiple points in day 1 (Zhu et al., 2008). Therefore, the difference in sampling points may contribute to the difference in Vdss between Japanese and non-Japanese CHB patients. Also, imprecision in estimation of Q/F may generate the difference of V3/F and Vdss. Population mean (95% confidence

interval) of Q/F in this population analysis in Japanese CHB patients was 913 (−37 to 1863). The wide confidence interval indicated the imprecise estimation of Q/F and, therefore, further comparison of Q/F between 2 populations was not implemented.

4. Discussion

The characterization of the population PK of ETV in Japanese CHB patients, and the comparison of ETV exposures in Japanese and non-Japanese CHB patients reported in this article were used to bridge the safety and efficacy of ETV established in the Japanese and global clinical programs, and enabled the data collected in the Japanese ETV program to be interpreted in the context of the larger global clinical database. Furthermore, the pooled model-based analysis of the PK data from Japanese and non-Japanese CHB patients enabled a quantification of the effect of ethnicity on ETV PK model parameters, after accounting for the effect of covariate–parameter relationships; an effect that may have otherwise been confounded.

ETV concentration–time data from Japanese CHB patients were well described by a 2-compartment model with first-order elimination, which was identical in structure to the population PK model used to describe ETV concentration–time data from non-Japanese CHB patients (Matthews, 2006). Moreover, the covariate–parameter relationships identified previously in the non-Japanese population PK analysis were also found to be significant for the Japanese CHB patients. Specifically, this analysis provides an independent confirmation of the previous findings that CL/F increases with increases in ICrCL and that Q/F decreases with increasing dose (Zhu et al., 2008). The stability of these covariate–parameter relationships was rigorously tested and confirmed by bootstrap and permutation test analysis of the Japanese data.

The covariate–parameter relationship between CL/F and ICrCL is consistent with data from phase I studies showing that renal elimination is the primary pathway of ETV clearance (Amano and Goto, 2007; Ishikawa, 2006; Matthews, 2006). ETV is not appreciably protein bound (13%) (Matthews, 2006), suggesting that the observation of dose effect on Q/F is not related to saturable protein binding. ETV showed nonlinear PK after single dosing at the range of 0.05–2.5 mg, but linear PK at steady state in a multiple dose (MAD) study (Yan et al., 2002). In addition, ETV has high oral bioavailability (>70%) (Matthews, 2006), and its metabolism does not significantly contribute to the elimination (Matthews, 2006). Furthermore, the percentage urinary recovery increased with increasing doses after single dosing at the range of 0.1–1.0 mg (unpublished data). These data suggested saturable peripheral distribution of ETV and, therefore, the covariate–parameter relationship was observed between Q/F and ETV dose. Given the nonlinear PK after a single dose and the dose-dependent intercompartmental clearance, data from future studies will be useful to develop mechanistic PK models to explain these observations. In addition, the available data did not support inclusion of the effects of age or gender on ETV PK parameters in either the non-Japanese or the Japanese population PK analyses (Zhu et al., 2008).

Although the median AUC_{ss} of Japanese patients at 1.0 mg is lower than that of non-Japanese patients by approximately 20%, this difference is likely to be due to the higher ICrCL in Japanese patients and not due to an effect of ethnicity on CL/F. The median ICrCL in Japanese patients who received the 1.0-mg dose is approximately 20% higher than the value in non-Japanese patients who received this dose. A pooled analysis of the Japanese and non-Japanese data confirmed that ethnicity does not have a statistically significant or clinically meaningful effect on ETV CL/F, indicating that the ETV exposure of Japanese patients is similar to that of non-Japanese patients after accounting for the effect of ICrCL. Nonetheless, similarities in dose–response (reduction in HBV DNA level) of Japanese and non-Japanese patients in studies AI463047 and AI463005 (Amano and Goto, 2007; Ishikawa, 2006; Lai et al., 2002) demonstrate that the differences in ETV AUC_{ss} are not clinically meaningful and that no adjustment of the ETV dose is warranted for Japanese CHB patients.

In conclusion, the PK of ETV in Japanese CHB patients is consistent with the previously characterized PK of ETV in non-Japanese CHB patients (Zhu et al., 2008). This report illustrates that a pooled model-based covariate analysis is useful to evaluate whether observed differences in ETV exposure between 2 populations (such as Japanese and non-Japanese CHB patients) are explainable by an effect of ethnicity or differences in other covariates known to affect the exposure of ETV. The results of this pooled model-based analysis provided data to support the bridging strategy for ETV. Approval of ETV for the treatment of adult patients with CHB was granted by the Japanese Ministry of Health, Labor, and Welfare in 2006.

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References

Amano M, Goto A (2007) [Pharmacological properties and clinical efficacy of entecavir monohydrate (Baraclude Tablet 0.5 mg), an anti-HBV drug]. *Nippon Yakurigaku Zasshi* 129:287–297.

Chang TT, Gish RG, Hadziyannis SJ, Cianciara J, Rizzetto M, Schiff ER, Pastore G, Bacon BR, Poynard T, Joshi S, Klesczewski KS, Thiry A, Rose RE, Colonno RJ, Hindes RG (2005) A dose-ranging study of the efficacy and tolerability of entecavir in lamivudine-refractory chronic hepatitis B patients. *Gastroenterology* 129:1198–1209.

Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41.

De Man RA, Wolters LM, Nevens F, Chua D, Sherman M, Lai CL, Gadano A, Lee Y, Mazzotta F, Thomas N, Dehertogh D (2001) Safety and efficacy of oral entecavir given for 28 days in patients with chronic hepatitis B virus infection. *Hepatology* 34:578–582.

Devine BJ (1974) Gentamicin therapy. *Drug Intell Clin Pharm* 8:650–655.

Ette EI (1997) Stability and performance of a population pharmacokinetic model. *J Clin Pharmacol* 37:486–495.

Good P (2000) Permutation test: a practical guide to resampling methods for testing hypotheses. Heidelberg: Springer.

Ishikawa H (2006) Entecavir monohydrate (Baraclude Tablet 0.5mg), an anti-HBV drug. *JSHP* 42:1636–1638.

Jonsson EN, Karlsson MO (1998) Automated covariate model building within NONMEM. *Pharm Res* 15:1463–1468.

Kobashi H, Takaguchi K, Ikeda H, Yokosuka O, Moriyama M, Imazeki F, Kage M, Seriu T, Omata M, Sakaguchi K, Shiratori Y (2009) Efficacy and safety of entecavir in nucleoside-naive, chronic hepatitis B patients: phase II clinical study in Japan. *J Gastroenterol Hepatol* 24:255–261.

LaCreta F, Mould D, Bifano M, Grasela DM, Pfister M (2005) Simulation based support of dose recommendation of entecavir for renal impaired subjects. *Clin Pharm Ther* 77:20 (Abstract PI-45).

Lai CL, Rosmawati M, Lao J, Van Vlierberghe H, Anderson FH, Thomas N, Dehertogh D (2002) Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. *Gastroenterology* 123:1831–1838.

Matthews SJ (2006) Entecavir for the treatment of chronic hepatitis B virus infection. *Clin Ther* 28:184–203.

Ogata Y (2007) In: *Clinical Pharmacokinetics*. Ogata H, Ed. Tokyo: Maruzen Co, Ltd, pp. 101–104.

Parke J, Holford NH, Charles BG (1999) A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. *Comput Methods Programs Biomed* 59:19–29.

Seifer M, Hamatake RK, Colonno RJ, Standring DN (1998) In vitro inhibition of hepadnavirus polymerases by the triphosphates of BMS-200475 and lobucavir. *Antimicrob Agents Chemother* 42:3200–3208.

Suzuki F, Toyoda J, Katano Y, Sata M, Moriyama M, Imazeki F, Kage M, Seriu T, Omata M, Kumada H (2008) Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. *J Gastroenterol Hepatol* 23: 1320–1326.

Tsuchiwata S, Mihara K, Yafune A, Ogata H (2005) Evaluation of Bayesian estimation of pharmacokinetic parameters. *Ther Drug Monit* 27:18–24.

Winter ME (2003) Basic clinical pharmacokinetics. Philadelphia: Lippincott Williams & Wilkins, pp. 100.

Yan JH, Bifano M, Nichola P, Mara EO (2002) Entecavir pharmacokinetics after multiple doses in healthy subjects. *J Clin Pharmacol* 42:1070.

Zhu M, Bifano M, Xu X, Wang Y, Grasela DM, Pfister M (2008) Lack of an effect of HIV co-infection on the pharmacokinetics of entecavir in HBV infected patients. *Antimicrob Agents Chemother* 52:2836–2841.