Food effect on the pharmacokinetics of entecavir from dispersible tablets following oral administration in healthy Chinese volunteers

Qing-Hua Zhang¹, Jin Yang¹, Ying He¹, Fang Liu², Ji-Ping Wang³, Andrew K. Davey³

¹ Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing (P. R. China)

² Jiangsu Province Hospital of TCM, Nanjing (P. R. China)

³ Sansom Institute, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA (Australia)

Correspondence to: Prof. Jin Yang, PhD, Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, 24# Tong Jia Xiang, Nanjing, P. R. China; email: yangjingqw@263.net

Key words

- Antiviral agents
- CAS 142217-69-4
- Entecavir, dispersible tablets, food effects, pharmacokinetics

Arzneimittelforschung
2010;60(10):640–644

Abstract

Objective: The aim of the present study was to assess the effect of food on the pharmacokinetics of entecavir (CAS 142217-69-4) from dispersible tablets.

Methods: In an open-label, two-way crossover study, 12 healthy Chinese volunteers randomly received a single oral dose of 1 mg entecavir dispersible tablets under fasted and fed conditions. Blood samples were collected and determined for pharmacokinetic analyses. A solid phase extraction for sample preparation and a LC/MS method were developed and validated for determination of entecavir in human plasma.

Results: The absorption of entecavir from dispersible tablets was altered significantly with food intake, as evidenced by a decrease in C_{max} of 63 %, a decrease in AUC_{0-1} of 22 %, and a delay in T_{max} of 1.5 h. The calibration curve was linear from 0.2 to 25 ng/mL, with a lower limit of quantitation (LLOQ) of 0.2 ng/mL.

Conclusion: Food intake has an obvious effect on the absorption of entecavir from dispersible tablets. It is better to take entecavir dispersible tablets on an empty stomach.

1. Introduction

Chronic hepatitis B virus (HBV) infection affects more than 350 million people worldwide [1] and is highly endemic in China [2]. According to the seroepidemiological survey on HBV infection conducted in 2006, about 30 million patients were chronic hepatitis B carriers among 93 million HBV carriers [3, 4]. Seven antiviral agents have been approved for the treatment of chronic hepatitis B currently, including interferon α -2a, lamivudine, pegylated interferon α -2a, adefovir, tenofovir, telbivudine, and entecavir [5]. Entecavir, a guanosine nucleoside analogue, has potent and selective activity against HBV with few side effects or mitochondrial toxicity. Importantly, it has a low risk of resistance and can be an alternative in lamivudine-refractory patients [6–9]. In addition, entecavir is likely to be more cost-effective than lamivudine in treating hepatitis B patients in China [10].

Recently, a new dosage form of entecavir, dispersible tablet, was developed and put into clinical trials. Dispersible tablets have a short disintegration time and are convenient for patients who need dosage regulation, especially for those who have difficulty in swallowing. Our previous study which was conducted in March 2009 at the Department of Clinical Pharmacology, Jiangsu Province Hospital of Traditional Chinese Medicine, in 20 healthy Chinese volunteers has shown that entecavir dispersible tablets are bioequivalent to normal tablets (not published). The influence of food on the absorption of entecavir tablets was shown in the label that after a standard high-fat meal (945 kcal, 54.6 g fat) or a light meal (379 kcal, 8.2 g fat), the absorption of 0.5 mg entecavir was delayed (1.0–1.5 h fed *vs.* 0.75 h fasted), the AUC and C_{max} were decreased by 18–20 % and 44–46 % respectively, hence entecavir should be administered at least 2 h after a meal and 2 h before the next meal. However, there is no report about the

food effect on the bioavailability of entecavir dispersible tablets. This study was conducted to investigate the possible pharmacokinetic differences of entecavir in fed and fasted volunteers given 1 mg entecavir from dispersible tablets following oral administration.

2. Subjects and methods

2.1 Subjects

Six male and 6 female healthy Chinese volunteers, between 20 and 28 years old, with a body weight index from 19 to 24 kg/m² were enrolled in the study. All subjects received an evaluation of medical history, physical examination, electrocardiograms and clinical laboratory examination. Those who had a history of, or were suffering from, serious acute or chronic illness, gastrointestinal disease within 3 months, any major surgery or any gastrointestinal surgery within 4 weeks of enrollment, blood transfusion within 4 weeks, and drug or alcohol abuse within 6 months were excluded. Subjects were also excluded if their blood examination for hepatitis B surface antigen was positive. All female subjects were non-pregnant, not planning pregnancy and non-breast-feeding. Written informed consent was obtained from all subjects before the start of the study and the protocol was approved by the Ethical Committee of Jiangsu Province Hospital of Traditional Chinese Medicine.

2.2 Study design

This was a single-dose, open-label, randomized, 2-period, cross-over study conducted from March 26, 2009 to April 28, 2009 at the Department of Clinical Pharmacology, Jiangsu Province Hospital of Traditional Chinese Medicine. Twelve healthy volunteers randomly received a single dose of 1 mg entecavir dispersible tablets (provided by Hainan Zhonghe Pharmaceutical Co., Ltd, Haikou, P. R. China, only for the clinical trial, batch number: 20081105, expiry date: November 5, 2009) as 2 × 0.5 mg tablets under fasted and fed conditions with a washout period of 28 days. The fasting group was fasted for 12 h prior to dosing, and allowed to take food 4 h after dosing, while the fed group was administered entecavir dispersible tablets immediately following a meal consisting of 2 eggs, 200 mL milk and 2 pork buns (1060 kcal, 68 g fat). Drug was administered with 250 mL water.

2.3 Blood sample collection

Blood samples (5 mL) were collected into heparinized tubes prior to and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 12, 15 and 24 h after dosing. Plasma samples were separated by centrifugation and stored at -26 °C until analysis.

2.4 Plasma sample analysis

Concentrations of entecavir in the plasma samples were determined using a validated LC/MS method with a solid phase extraction pretreatment. Briefly, 10 µL internal standard (IS, ganciclovir) and 600 µL 10 % perchloric acid were added to 1 mL plasma. After vortexing for 1 min and centrifuging at 20,627 g for 5 min the supernatant (700 µL) was loaded on an Oasis® MCX extraction column (Waters Corporation, Milford, MA, USA) which was pretreated with 1 mL methanol and 1 mL water. After gravity elution, the column was washed with a 1 mL mixture of water and formic acid (98:2) and 1 mL methanol, and then the eluate was discarded. Subsequently, the col-

umn was washed with 1 mL mixture of ammonia and methanol (96:4) and the eluate was collected into a 10 mL glass centrifuge tube. The eluate was dried under air flow in a 45 °C water bath and the residue redissolved in mobile phase A. After vortexing, the sample was transferred into a microcentrifuge tube and centrifuged at 20,627 g for 5 min. Five µL supernatant was injected into the LC/MS system for analysis.

An LC/MS 2010 system (Shimadzu, Kyoto Prefecture, Japan) comprising two LC-20AD pumps, a CBM-20A system controller, a CTO-20A column oven, a SIL-20AC auto sampler and a single quadrupole mass spectrometer equipped with an electrospray ionization interface was used for analysis. Separation was carried out on a Lichrospher RP C18 column (150 × 2.0 mm, 5 µm) (Hanbon Sci. & Tech., Jiangyin, P. R. China) and the oven temperature was set at 35 °C. Gradient elution was performed with 0.02 % formic acid in water as mobile phase A and methanol as mobile phase B at a flow rate of 0.2 mL/min. One min after sample injection, the percentage of mobile phase B was increased from 13 % to 35 % over 3 min. Then that was decreased from 35 % to 13 % in 0.01 min and retained at 13 % for 5 min. Selected ion monitoring (SIM) was used to detect entecavir and ganciclovir in positive mode at mass/charge ratio (*m/z*) 277.9 and 256, respectively. Operating parameters for electrospray ionization mass spectrometry included capillary voltage 1.5 kV, nebulizer nitrogen flow rate 1.5 L/min, and drying gas temperature 250 °C.

2.5 Safety and tolerability assessment

Safety and tolerability were assessed according to the adverse events (AEs) reported by the investigators and volunteers, based on the clinical laboratory examination, electrocardiogram, physical examination, and vital signs. AEs were evaluated in terms of severity (mild, moderate, severe or life threatening) and relationship to study treatment (definite, probable, possible, doubtful, highly unlikely).

2.6 Pharmacokinetic analysis

PK parameters of entecavir were calculated using BAPP 3.2 (Bioavailability Program Package 3.2, Yangjin, Nanjing, P. R. China) [11]. The maximum plasma concentration (*C*_{max}) and the time to achieve *C*_{max} (*T*_{max}) were obtained directly from the plasma concentration-time curves. The area under the plasma concentration-time curve from 0 to the terminal sampling time (*AUC*_{0-*t*}) was calculated using the linear trapezoidal rule. The drug-elimination half-life (*T*_{1/2}) was calculated using the terminal phase. The geometric mean ratio (GMR) of *AUC*_{0-*t*} (fed) to *AUC*_{0-*t*} (fast) was used to calculate the relative bioavailability (*F*) and 90 % confidence interval (90 % CI) was obtained.

2.7 Statistical analysis

*C*_{max} and *AUC*_{0-*t*} were natural logarithm transformed, and evaluated using analysis of variance and two-tailed *t* test. *P* values lower than 0.05 were accepted as statistically significant. *T*_{max} was analyzed using a Wilcoxon pairwise signed rank test. Statistical analysis was performed using the BAPP 3.2 statistical software.

Fig. 1

3. Results

3.1 Assay validation

Chromatograms are shown in Fig. 1. The assay was free of interference and retention times of entecavir and ganciclovir were 2.9 and 6.3 min respectively. The calibration curve was linear in the range 0.2 to 25 ng/mL with a lower limit of quantification (LLOQ) of 0.2 ng/mL.

Fig. 1: Mass spectrometry chromatograms of (I) entecavir and (II) ganciclovir (internal standard) in human plasma. (A) Blank plasma; (B) blank plasma spiked with entecavir (1 µg/mL) and IS; (C) plasma sample collected at 0.5 h after a single oral dose of 1 mg entecavir dispersible tablets.

Table 1: Precision and accuracy for the determination of entecavir in human plasma (n = 5).

Nominal conc. (ng/mL)	Mean found conc. (ng/mL)	Intra-day RSD (%)	Inter-day RSD (%)
0.5	0.502	5.0	7.5
5	4.98	4.0	1.1
20	19.52	3.2	4.6

RSD: relative standard deviation.

Precision, recovery, matrix effect and stability were satisfactory at 3 different concentration levels (0.5, 5 and 20 ng/mL, quality control (QC) samples). Intra-day and inter-day relative SD (RSD) values (Table 1) were all $\leq 7.5\%$. Recoveries during the sample extraction at 3 different concentration levels were 91.7%, 90.6% and 90.1%, respectively. Matrix effects of entecavir were considered to be moderate (95.9% to 111.7%), whereas that of the IS were 119.8% to 121.4%, which indicates a small but consistent effect. Stability was evaluated in QC samples stored for 24 h at room temperature, 1 month at -26°C and subjected to 3 freeze-thaw cycles. Entecavir was stable under all of the storage conditions and mean recoveries were 89.7% to 102.6%.

3.2 Food effect on the pharmacokinetics of entecavir

As shown in Fig. 2, mean plasma pharmacokinetic profiles of 1 mg entecavir dispersible tablets administered

Fig. 2: Mean plasma concentration-time profiles of entecavir after a single oral dose of 1 mg entecavir dispersible tablets in healthy Chinese volunteers under fasted and fed conditions.

Table 2: Summary of entecavir pharmacokinetic parameters in healthy Chinese volunteers after a single oral dose of 1 mg entecavir dispersible tablets under fasted and fed conditions (n = 12, mean ± SD).

Parameters	Fasted	Fed
C_{max} (ng/mL)	11.21 ± 3.76	4.15 ± 1.68**
T_{max} (h)	0.75 (0.25–0.75)*	2.5 (0.25–5)**
$t_{1/2}$ (h)	66.29 ± 87.31	39.23 ± 25.13
AUC_{0-t} (ng · h/mL)	27.23 ± 4.54	21.14 ± 4.69**
$F(\%)_{GMR}$, 90 % CI	77.7** [66.2–88.7]	

* Median (minimum–maximum). ** $P < 0.05$.

orally with and without food are obviously different. Compared to the fasted group, a high-fat meal caused a decrease in C_{max} of 63 %, a decrease in AUC_{0-t} of 22 %, and a delay in T_{max} of 1.5 h as summarized in Table 2. The relative bioavailability (F) is 77.7 [66.2–88.7] %. Therefore, the intake of food altered the pharmacokinetics of entecavir from dispersible tablets significantly.

3.3 Safety evaluation

No serious AEs occurred during the study, and no clinically meaningful changes appeared during the physical examinations. Only 3 mild AEs were reported. One participant suffered from an upper respiratory infection which was judged unrelated to entecavir. The other two cases of blood system involvement were considered to be doubtfully related to the study treatment.

4. Discussion

This study utilized a simple, selective and validated LC/MS assay based on selected ion monitoring rather than a more technically demanding LC-MS/MS technique [12]. The sensitivity was adequate for PK studies of entecavir dispersible tablets. During the assay development,

we recognized that entecavir is a small and polar compound that is not ideally suited for reverse phase liquid-liquid extraction, so we developed a solid phase extraction method using Waters Oasis® MCX columns which are specific for weak bases. Results showed that this sample pretreatment procedure can remove most endogenous compounds with high recoveries and low matrix effect. In terms of chromatography, we used a simple mobile phase containing 0.02 % formic acid and methanol by gradient elution to achieve adequate retention of entecavir and ganciclovir and better ionized efficiency.

According to the physicochemical properties of entecavir, it is a weak base belonging to BCS III drugs (BCS: Biopharmaceutics Classification System) which have good solubility but poor permeability [13]. As a result of the good solubility of entecavir, absorption from dispersible tablets in the gastrointestinal tract was not superior to the standard tablets and the influence of food on them is similar. The decrease in C_{max} and prolongation of T_{max} are likely due to food delaying the stomach emptying [14]. Another possible reason is that, with the increasing of gastric pH following the high-fat meal, the dissolution rate of entecavir will be reduced.

A high-fat meal decreases the rate and the extent of oral absorption of entecavir. This phenomenon was also observed in the pharmacokinetic studies of some other nucleoside drugs, such as didanosine, zidovudine, lamivudine, zalcitabine, stavudine and abacavir. The C_{max} and AUC_{0-t} values of these drugs were lower and T_{max} was delayed after a meal [15]. Further study on didanosine showed that food impact on the decrease of AUC was similar regardless of food composition, which suggested that the gastric emptying is not the direct reason for the reduction of AUC [15]. The current published studies are insufficient to explain the reason of food effects on nucleoside drugs, especially on the loss of bioavailability.

It should be noted that when administered with a meal, the bioavailability of most drugs which do not have a specific absorption site in the intestinal tract is not changed. Conversely, drugs that are only well absorbed in the upper intestine show a decreased absorption when administered with food due to effects such as precipitation, binding interactions and increased viscosity which restricts drug diffusion [16]. Therefore, some effects that reduce the absorption of drug in this region independent of the meal content may be critical [16]. Many drugs belonging to BCS III show region dependent absorption, with a better absorption in the upper small intestine [16, 17]. We have conducted a study on the absorption of entecavir in excised rat intestine segments in the Ussing Chamber recently (unpublished), and the value of P_{app} in duodenum, jejunum and ileum was 6.42 ± 2.63 , 4.80 ± 3.97 , $2.71 \pm 0.71 \times 10^{-6}$ cm/s respectively. This suggests that intestinal permeability of entecavir is site dependent, with a better absorption in the upper intestine. Results of the mechanism study on entecavir intestinal absorption in rats described above indicated that food impeded the access of entecavir to the epithelium surface of the intestine thus decreasing its bioavailability [14, 16]. This finding may provide a reasonable explanation for the food impact on the absorption of entecavir, but the exact mechanism still needs further investigation.

According to the report of Yan *et al.* [18], the plasma concentrations of entecavir had a minor descending trend from 24 h to day 7 after a single dose, so we set the terminal sampling time at 24 h. In our experiment, terminal mean plasma concentration was about 1/40 of the mean C_{max} values, which is in accordance with the FDA guidance. Taking account of the circadian rhythms, chronopharmacokinetics and the detecting error, the terminal elimination half-life in our study was shorter than the value in the label and the dose-escalation study (128–149 h) [6, 18], with a large coefficient variance of 132% and 64% under fasted and fed conditions, respectively. Therefore, $T_{1/2}$ is not suggested to be applied in the dosage regimen.

In conclusion, this study demonstrated that food effect on the pharmacokinetics of entecavir from dispersible tablets was similar to that of the existing dosage form with regard to C_{max} , T_{max} , and AUC_{0-t} . It is recommended that entecavir dispersible tablets should be taken in the absence of food for optimal bioavailability.

Acknowledgement

This work was supported by Hainan Zhonghe Pharmaceutical Co., Ltd, Haikou (P. R. China).

Conflict of Interest

There are no competing interests for all authors involved.

References

- [1] Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet*. 2009;373:582–92.
- [2] Liang XF, Chen YS, Wang XJ, He X, Chen LJ, Wang J, *et al.* A study on the seroepidemiology of hepatitis B in Chinese population aged over 3-years old: the report from Chinese Center for Disease Control and Prevention. *Chin J Epidemiol*. 2005;26:655–8.
- [3] Lu FM, Zhuang H. Management of hepatitis B in China. *Chin Med J*. 2009;122:3–4.
- [4] Data on 2006 seroepidemiological survey on HBV infection in China. Available from: <http://www.moh.gov.cn>
- [5] Shamliyan TA, MacDonald R, Shaukat A, Taylor BC, Yuan JM, Johnson JR, *et al.* Antiviral therapy for adults with chronic hepatitis B: A systematic review for a National Institutes of Health Consensus Development Conference. *Ann Intern Med*. 2009;150:111–24.
- [6] Baraclude™ (entecavir) package insert. Princeton (NJ): Bristol-Myers Squibb Company. March 2005.
- [7] Santantonio T, Fasano M. Resistance profile of entecavir in patients with chronic hepatitis B. *Dig Liver Dis*. 2008; Suppl 2: 11–5.
- [8] Colonna RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, *et al.* Entecavir resistance is rare in nucleoside naive patients with hepatitis B. *Hepatology*. 2006;44:1656–65.
- [9] Yao GB, Zhou XQ, Xu DZ, Wang BE, Ren H, Liu J, *et al.* Entecavir for the treatment of lamivudine-refractory chronic hepatitis B patients in China. *Hepatol Int*. 2007;1:373–81.
- [10] Yuan Y, Illoeje U, Li H, Hay J, Yao GB. Economic implications of entecavir treatment in suppressing viral replication in chronic hepatitis B (CHB) patients in china from a perspective of the Chinese Social Security Program. *Value Health*. 2008; Suppl II: S11–22.
- [11] Yang J, Liu XQ, Liu XD. Development of a bioavailability program package 2.0. *Chin J Clin Pharmacol*. 2003;19:125–7.
- [12] Zhang DX, Fu YL, Gale JP, Aubry AF, Arnold ME. A sensitive method for the determination of entecavir at picogram per milliliter level in human plasma by solid phase extraction and high-pH LC-MS/MS. *J Pharm Biomed Anal*. 2008;49(4): 1027–33.
- [13] Hivid (zalcitabine) Nutley, NJ: Hoffman-La Roche. 2002. Physician's Desk Reference Electronic Library. 2004.
- [14] Gu CH, Li H, Levons J, Lentz K, Gandhi RB, Raghavan K, *et al.* Predicting effect of food on extent of drug absorption based on physicochemical properties. *Pharm Res*. 2007;24:1118–30.
- [15] Damle BD, Yan JH, Behr D, O'Mara E, Nichola P, Kaul S, *et al.* Effect of food on the oral bioavailability of didanosine from encapsulated enteric-coated beads. *J Clin Pharmacol*. 2002;42:419–27.
- [16] Fleisher D, Li C, Zhou YJ, Pao LH, Karim A. Drug, meal and formulation interactions influencing drug absorption after oral administration: clinical implications. *Clin Pharmacokinet*. 1999;36:233–54.
- [17] Pao LH, Zhou SY, Cook C, Kararli T, Kirchhoff C, Truelove J, *et al.* Reduced systemic availability of an antiarrhythmic drug, bidisomide, with meal co-administration: relationship with region-dependent intestinal absorption. *Pharm Res*. 1998;15(2):221–7.
- [18] Yan JH, Bifano M, Olsen S, Smith RA, Zhang DX, Grasela DM, *et al.* Entecavir pharmacokinetics, safety, and tolerability after multiple ascending doses in healthy subjects. *J Clin Pharmacol*. 2006;46:1250–8.