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Research Article

Determination of Pharmacokinetics and Pharmacodynamics of Lamivudine After Highdoses in Duck Hepatitis B Virus-Infected Pekin Ducks

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Abbreviations AUC: Area Under the Serum Concentration-Versus-Time Curve; cccDNA: Covalently Closed Circular Viral DNA; Clap: Apparent Total Body Clearance; DHBV: Duck Hepatitis B Virus; HBV: Human Hepatitis B Virus; HIV: Human Immunodeficiency Virus; IM: Intramuscular; IV: Intravenous; PO: Oral Administration; Vdap: Apparent Volume of Distribution

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Abstract

Purpose: The Duck Hepatitis B Virus (DHBV)-infected Pekin duck model has been shown to be a reference model for the evaluation of anti-HBV treatments. The purpose of the current study was to characterize the pharmacokinetic and pharmacodynamic profiles of lamivudine, a potent nucleoside inhibitor of HBV replication, in DHBV-infected Pekin ducks.

Methods: Lamivudine serum concentrations were measured by LC-ESI-MS/MS following the administration of 80mg/kg IV, 200 mg/kg IM and 480 mg/kg PO of lamivudine to DHBV-infected Pekin ducks. Whereas, DHBV viremia levels were measured by real-time-PCR before, during and after 6-week lamivudine treatment of 40mg/kg IM daily, or 100 or 200 mg/kg PO daily.

Results: The average apparent total body clearance and volume of distribution of lamivudine were 0.29 L/hr/kgand0.65 L/kg, respectively. The average area under the concentration-time curve was 318, 661 and 1344 µg.hr/mL for 80mg/kg IV, 200 mg/kg IM and 480 mg/kg PO of lamivudine, respectively. 6-week lamivudine treatment of 100 mg/kg and 200 mg/kg PO were indifferently able to significantly lower DHBV titers compared with control and 40 mg/kg IM groups. However, the latent suppression of DHBV titers after the termination of lamivudine treatment was significantly more in 200 mg/kg PO compared with 100 mg/kg PO.

Conclusions: Our results suggest that the optimum dose of lamivudine against chronic HBV is higher than the current recommended dose in human.

Introduction

It is estimated that approximately two billion individuals have been infected with Hepatitis B Virus (HBV) at some point in their lives. Of these, over four hundred million develop chronic HBV infection, with persistent HBV DNA and HBV Surface Antigen (HBsAg) in their serum [1]. As a consequence of chronic HBV infection these patients are at high risk of developing cirrhosis, Hepatocellular Carcinoma (HCC), and liver failure resulting in over a million deaths each year [1,2]. The efficacy of current chronic HBV treatments, namely interferon-α and nucleoside analogues, is limited by the high barrier of drug-resistant mutants in addition to the persistence of intranuclear Covalently Closed, Circular Viral DNA (cccDNA), which is responsible for viral relapse as a consequence of treatment withdrawal [3-7].

There are six nucleoside analogues approved for the treatment of HBV, namely lamivudine, adefovir, entecavir, telbivudine, tenofovir and emtricitabine. Lamivudine [(-)-2′-3′-dideoxy-3′-thiacytidine] has been also approved for the treatment of human immunodeficiency virus (HIV) but at higher daily oral dose of 300 mg, compared with 100 mg for HBV. Lamivudine is phosphorylated inside the body to lamivudine-5′-triphosphate, which is a potent inhibitor of viral replication by acting as a chain terminator of viral DNA synthesis and also by competitively inhibiting viral reverse transcriptase [8-12]. In chronic HBV patients, there is a marked reduction in viremia during lamivudine treatment followed by a reversal of T cell anergy, resulting in enhancement of the HBV-specific cytotoxic T cell activity and CD8+ T-cell proliferation [13,14]. However, lamivudine has a low genetic barrier of resistance, placing lamivudine as an alternative line of chronic HBV treatment according to WHO guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection [15]. Several reports have argued that lamivudine dose has not been properly adjusted and increasing the daily dose of lamivudine to 300 mg would impair the development of

resistance and enhance the therapeutic outcome of lamivudine [16-18]. Though, lamivudine is still in the first-line of treatment in HBV/HIV-coinfected adults, adolescents and children [15].

While lamivudine has been used in different animal models for HBV, the duck hepatitis B virus (DHBV)-infected Pekinduck model has been shown to be a reference model for the evaluation of novel anti-HBV approaches and for testing their ability to clear viral cccDNA [19-22]. DHBV infection of newly hatched ducklings results in nearly 100% chronic infection, similar to neonatal HBV infection of humans. Therefore, this approach was used in the present study to establish a flock of chronic DHBV carriers of the same age. The objectives of the current study were first to characterize the pharmacokinetic profile of lamivudine in DHBV-infected Pekin ducks following Intravenous (IV), Intramuscular (IM) and Oral (PO) administration. The second objective was to quantify the effect of three lamivudine treatments of different pharmacokinetic/pharmacodynamic indices on DHBV titer in the serum of DHBV-infected Pekin duck.

Materials and Methods

Chemicals

Lamivudine was purchased from Royal Pharm (Hangzhou, China). The D4T (2′,3′-Didehydro-2′-deoxythymidine) compound that was used as an internal standard was purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile, methanol, formic acid, ammonium formate (High-Performance Liquid Chromatography [HPLC] grade) and other chemicals (analytical grade) were purchased from Fisher Scientific (Toronto, ON). Oasis HLB 1cc (30 mg) solid phase extraction cartridges were purchased from Waters (Mississauga, ON).

Animal studies

All experimental procedures involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee and performed in compliance with relevant institutional policies, the Association for the Accreditation of Laboratory Animal Care guidelines, the National Institutes of Health regulations and local, provincial and federal laws.

Infection of Ducklings with ClaI strain of DHBV

All animals were screened for the presence of DHBV infection by dot blotting prior to use in these studies. The mutant virus, DHBV-ClaI, was made as previously described [23]. ClaI DHBV strain contains a point mutation at nucleotide 1858 which introduces a ClaI restriction site without altering any amino acid sequence [23]. The adult Pekin ducks used in the current study were inoculated with the ClaI DHBV strain within 48 h of hatching, and the 100 µL of inoculum (1.10 x 109 genomic copies per mL) was injected intravenously in the medial metatarsal vein using insulin syringes. Animals were considered chronically infected if viremia was detected by PCR at 8 weeks after inoculation.

Experimental design

In the current study, two experiments were performed: 1) an experiment to determine the pharmacokinetic profile of lamivudine and 2) an experiment to investigate the effect of lamivudine administration on DHBV serum levels.

With respect to lamivudine pharmacokinetic profile, 10 adult DHBV-infected Pekin ducks (mean weight of 4.13 ± 0.35 kg) were used. Ducks were administered 80, 200 and 480 mg lamivudine per Kg of body weight by IV, IM and PO routes, respectively. An additional duck was administered saline served as a control. Blood samples were collected prior to drug administration and at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after dosing through venipuncture of the opposite medial metatarsal vein. The blood samples were centrifuged immediately after clotting at room temperature, and serum was stored at -80° C until analysis.

In the second experiment, 16 adult DHBV-infected Pekin ducks (mean weight of 4.13 ± 0.35 kg) were used. Groups of 4 ducks were treated with saline, 40, 100 or 200mglamivudine per Kg of body weight by IM or PO routes of administration for 6 weeks. Blood samples were collected 3-week before, during and 6-week after lamivudine treatment.

Measuring lamivudine serum concentration by LC-ESI-MS/MS assay

Serum samples obtained from ducks were first extracted by solid-phase extraction technique. Briefly, 10 µl of the internal standard (0.1 mg/ml of D4T) was added to 100 µl serum sample and an equal volume of methanol was added. After vortexing and centrifugation at 10,000 x g for 6 min, the supernatant was brought up to 1 mL with water. Oasis HLB cartridges were preconditioned with 1 mL of methanol and water, in sequence. Following that, cartridges were loaded and washed with 1 mL of 5% v/v methanol/water. Finally, samples were eluted twice with 1 mL of ice cold methanol, which was dried under vacuum, and re-suspended in 30 µl of 5% v/v methanol/water with 0.1% formic acid (pH~4.0). Chromatographic separation was achieved with a 150mm x 3.0mm Grace All tech Platinum column. The mobile phase consisted of 40mM NH4COOH in 1% acetonitrile pH 4.3 (buffer: A), and 100% acetonitrile (buffer: B). The flow rate of the mobile phase was 430 µl/min, and the gradient was 25%to 5% A in 10 min, 5% A to 100% Bin 1 min, then 100% B for 19 min. Mass spectrometry detection was carried out using Applied Bio systems mass spectrometer AB Sciex QTRAP 2000 coupled with the Agilent 1100 HPLC instrument. Multiple reaction monitoring under positive-ion mode was used, (230→112) for lamivudine and (225→127) for D4T. The standard curves were linear over the ranges of 2.9 to 857 mg/mL. Inter- and Intra-day accuracy (%error) and precision (coefficient of variation) were assessed at 2.9, 285.7, and 857 mg/ml, and they were less than 3.1% at all concentrations.

Quantitation of DHBV in duck serum

Serum (100 µL) was digested with 0.8 mg/mL proteinase K in 1% SDS and TSE Buffer, and incubated at 55°C for 2 h. DHBV DNA was then isolated using the Macherey-Nagel Nucleospin Gel & PCR Clean-up Kit. DHBV DNA titers were measured by qPCR using BioRad's CFX96 RT system, C1000 Thermal Cycler, with samples run in triplicate. Each reaction consisted of 2 µL of DNA sample, 10 µL of TaqMan® Universal Master Mix II (with UNG, from Applied Biosystems), 6 µL of 1.5 µM probe, and 2 µL of 1.5 µM of each primer. Primers used for DHBV were forward Primer: 5' - GGG AAA GGA GAG CC CTA CA - 3', reverse Primer: 5' -TCT ATG GTG GCT GCT CGA ACT - 3', probe: 5' - CCA ACG TGC GGG CTC CCC TC - 3' with a 5' FAM and 3' Iowa Black quencher. The protocol started

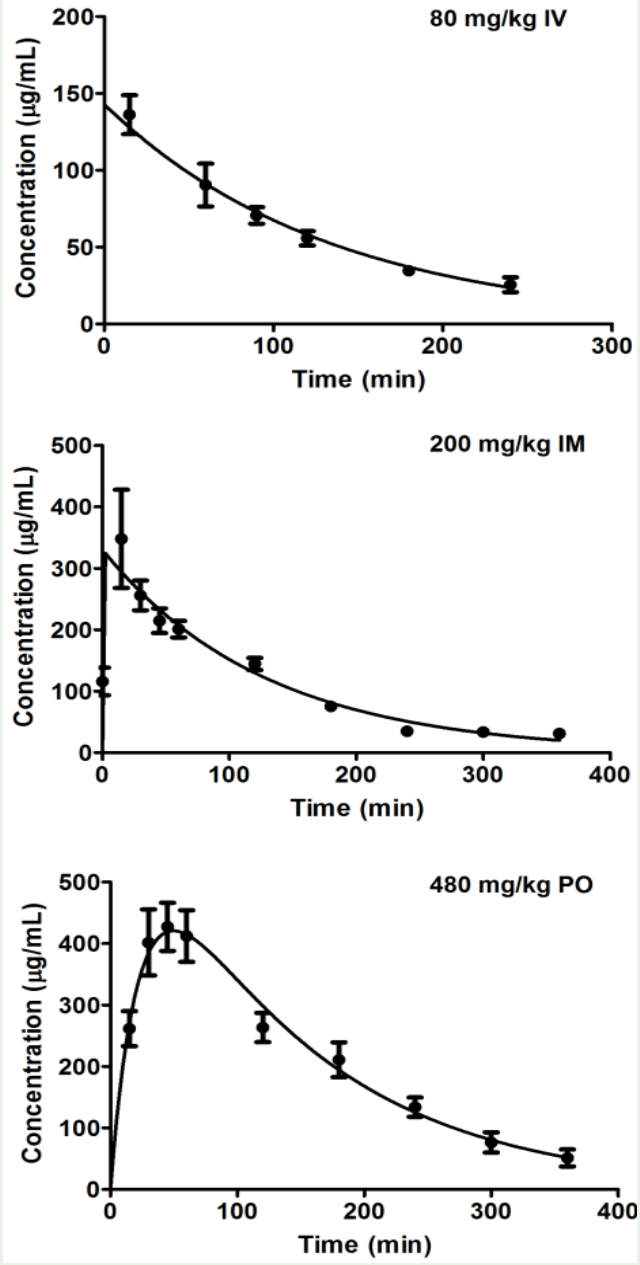

Figure 1: Concentrations of lamivudine in serum following single administration of 80mg/kg IV, 200 mg/kg IM and 480 mg/kg PO of lamivudine toDHBV-infected Pekin ducks. Results are presented as mean and SE (n=3).

at 45°C for 2 min (for UNG activity), 95°C for 10 min for hot-start activation of the DNA Polymerase, then 43 cycles of 95°C for 15 sec and 60°C for 30 sec for DNA denaturation and annealing/extension. To build the standard curve, pALT-DHBV16 monomer plasmid (generously provided by Dr. Qingxia Yao) was used as template. The plasmid was diluted to 109, 108,107, 106, 105, 104, 103, 102, 101 copies per µL.

Data analysis

Lamivudine serum concentration-time data fitting was performed using Graph Pad Prism (version 5.01; GraphPad Software, La Jolla, CA). One- and two-compartment models were tried, and optimal pharmacokinetic model was determined by the Akaike information criterion as a measure of the goodness of fit. The area under the serum concentration-versus-time curve (AUC) was calculated by linear-trapezoidal method. Absolute bioavailability was calculated as AUC*DoseIV/AUCIV*Dose. The C_{max} and T_{max} were determined from the original data. Lamivudine half-life (t1/2) was calculated as 0.693 x apparent volume of distribution (Vdap)/apparent total body clearance (Clap). The simulation of pharmacokinetic data and the calculation of pharmacokinetic/pharmacodynamics indices were performed using Wolfram Mathematica 10.1 (Wolfram Research, Inc., Champaign, IL). Regarding statistical analysis, one-way analysis of variance, followed by a Tukey's post hoc test, was used. A result was presented as mean±S.E and was considered statistically significant where $P < 0.05$.

Results and Discussion

Despite the common use of DHBV model to assess the efficiency of antivirals against human HBV, characterization of the pharmacokinetic profile of lamivudine in ducks has not been reported previously. Therefore, we determined, in DHBV-infected Pekin ducks, the values of pharmacokinetic parameters of lamivudine following IV, IM and PO administration of three escalating doses (80 to 480 mg/kg) to confirm the linearity of its pharmacokinetics. The serum concentration-time curves of the three routes are showed in figure 1. We found that lamivudine pharmacokinetics follows one-compartment kinetics, and pharmacokinetic parameters values and S.E. are shown in table 1. The elimination of lamivudine in ducks was remarkably higher than what was reported for human and woodchuck (t1/2 values were 1.5, 4.3 and 3.3 h, respectively) [24, 25]. However, our determined values for Clap and Vdap fitted perfectly in the reported allometric relationship with species body weight [25, 26]. The absorption of lamivudine after IM administration was significantly faster than after PO route (Tmax values were 0.32 and 0.58 h, respectively). Also, the extent of lamivudine absorption after IM administration was higher than after PO route (F values were 0.83 and 0.71 h, respectively). Our F values following PO administration are

Table 1: Pharmacokinetic parameters (mean±S.E.) after single dose of 80 mg/kg IV, 200 mg/kg IM and 480 mg/kg PO of lamivudine in Pekin ducks.

Route	Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (hr)	K_a (min-1)	Cl_{ap} (L/hr/Kg)	Vd_{ap} (L/Kg)	AUC (µg.hr/mL)	$t_{1/2}$ (hr)	F
IV	80	-	-	-	0.25±0.03	0.56±0.05	318±36.9	1.54±0.12	-
IM	200	351.5±76.5	0.25±0.07	431±101	0.28±0.03	0.61±0.05	661±58.1	1.48±0.11	0.83
PO	480	435±39.2	0.58±0.08	0.04±0.01	0.35±0.05	0.79±0.09	1344±258	1.57±0.14	0.71

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comparable to the reported human values (69-95%), albeit different from woodchuck values (18-54%). Human and duck HBV have several shared features that made Pekin ducks a successful model to provide insights into anti-HBV therapy [27]. Another avian HBV model is the Woodchuck Hepatitis Virus (WHV) model. Nevertheless, DHBV model has a clear advantage over WHV model because woodchucks undergo winter hibernation [28-30] and thus are impractical given the long duration of vaccine studies [31]. Hibernation also results in lower cardiac output that would possibly alter hepatic and renal blood flows [31], thus, measurement of pharmacokinetic parameters for a specific drug in woodchucks is a challenging task [25]. Consequently, there would be two different pharmacokinetic parameters to account for depending on the time of the year at which these studies were conducted. In either woodchucks or Pekin ducks, 20-40 mg/kg daily of lamivudine are believed to be equivalent to doses administered to human [23,32-34]. Our results showed that the serum concentration in ducks after 40 mg/kg of lamivudine will be approximately 10-fold higher than in human after lamivudine dose of 100 mg daily [24]. However, it has been reported that the IC50 against wild-type DHBV is approximately 12 fold higher than against wild-type human HBV [35,36]. Accordingly, we confirm that 40 mg/kg of lamivudine given IM to ducks is more or less equivalent to 100mg PO dose in humans.

In order to study the pharmacodynamics of lamivudine against DHBV, we investigated the effect of the usual IM dose of 40 mg/kg, as well as high daily PO doses of 100 and 200 mg daily of lamivudine on DHBV decay in vivo. The concentration-time curves of 40 mg/kg IM, and 100 and 200 mg/kg PO daily for 6 weeks were simulated, and are shown in figure 2A. In addition, the pharmacokinetic/pharmacodynamics indices of the three lamivudine treatments for the 6-week duration were calculated (Table 2). Our results showed that both 100 mg/kg and 200 mg/kg treatments were able to significantly suppress but not eliminate DHBV titers at all time points compared with either control or 40mg/kg groups (Figure 2B). A plateau of DHBV titers was reached by the fourth week of lamivudine treatment in case of 100 and 200 mg/kg doses, whereas, it was apparently reached by the second week for 40 mg/kg dose (Figure 2B). There was no significant difference in DHBV titers after 200 mg/kg compared with 100 mg/kg of lamivudine at any time point (Figure 2B). Interestingly, after 6 week of the termination of lamivudine treatment, 200 mg/kg dose led to a significantly lower serum DHBV titers compared with 100 mg/kg dose (Figure 3). With respect to the pharmacokinetic/pharmacodynamics indices, the ~2-fold increase in lamivudine dose from 40 to 100 to 200 mg/kg led to small increase in the total time the serum concentration of lamivudine was above the IC50 value of 0.1 μM (36) (t >IC50); during the 6-week duration of treatment t >IC50 was 706, 830, and 896 hr for 40, 100 and 200 mg/kg, respectively, which is correlated with the observed direct effect of lamivudine on DHBV titer (Table 2). In contrast, the AUC and C_{max} showed ~2-fold increase by increasing lamivudine dose from 40 to 100 to 200 mg/kg, which is more correlated with the observed latent effect of lamivudine on DHBV titer (Table 2).

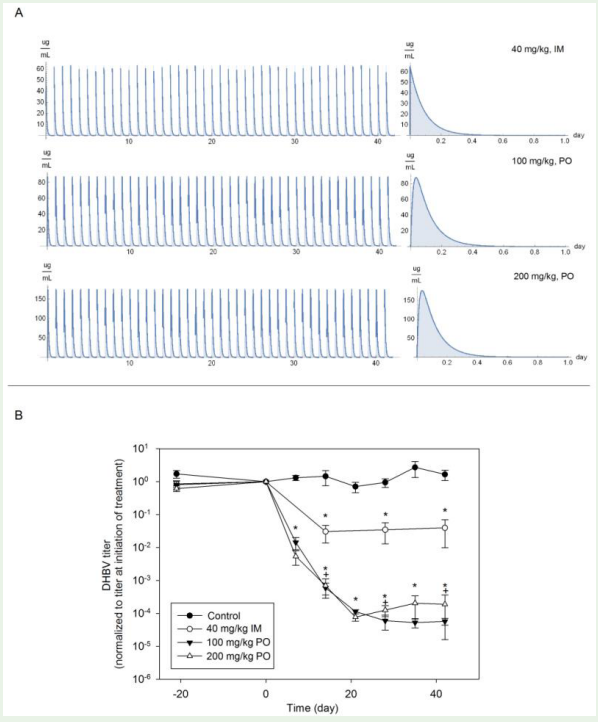

Figure 2: A; Simulation of concentration-time curves of single dose and 6-week treatment of lamivudine of 40mg/kg IM, or 100 mg/kg or 200 mg/kg PO daily toDHBV-infected Pekin ducks.
B; Viremia levels in Pekin ducks before and following 40mg/kg IM, or 100 mg/kg or 200 mg/kg PO daily of 6-week lamivudine treatment to DHBV-infected Pekin ducks. Results are expressed as DHBV DNA copies in serum normalized to the baseline DHBV DNA copies before treatment, and are presented as mean and SE (n=4).

In summary, the current study is the first to report the pharmacokinetic and pharmacodynamic profiles of lamivudine in DHBV-infected Pekin ducks. Lamivudine still has a clear cost advantage over the other competitors. In the current situation of continuous emerging resistance against lamivudine as well as its competitors, the clinical dose of lamivudine has to be adjusted to get the maximum efficiency out of it. Our results came to support previous reports suggesting that the optimum dose of lamivudine against chronic HBV is higher than the current recommended dose (16-18). 600 mg PO daily of lamivudine was previously reported to be tolerable in human [24]. Therefore, giving 600 mg daily in

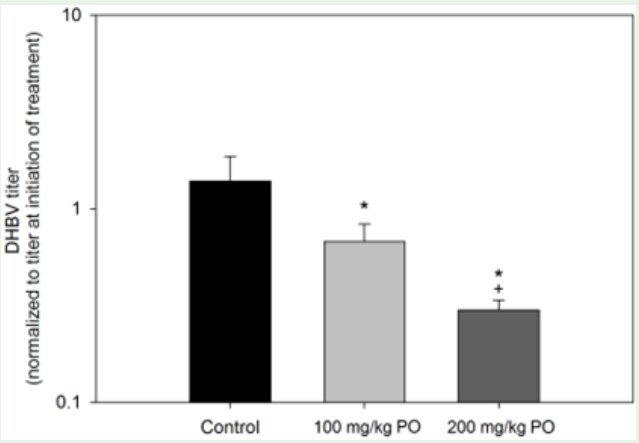

Figure 3: Viremia levels in Pekin ducks after 6 weeks of the termination of 100 mg/kg or 200 mg/kg PO daily of lamivudine treatment to DHBV-infected Pekin ducks. Results are expressed as DHBV DNA copies in serum normalized to the baseline DHBV DNA copies before treatment, and are presented as mean and SE (n=4).

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Table 2: The pharmacokinetic/pharmacodynamic indices of 40 mg/kg IM, and 100 and 200 mg/kg OP once daily for 6 weeks in Pekin ducks.

Route	Dose (mg/kg)	Week	Cumulative AUC (µg.hr/mL)	Cmax (µg/mL)	Cumulative t>IC50 (hr)	% residual DHBV titer
IM	40	1	977	65.1	118	ND
		2	1954		235	3.045
		3	2931		353	ND
		4	3908		471	3.460
		5	4885		589	ND
		6	5862		706	3.952
PO	100	1	2009	87.9	138	1.425
		2	4018		276	0.060
		3	6028		414	0.011
		4	8037		552	0.006
		5	10046		690	0.005
		6	12055		830	0.006
	200	1	4018	176	149	0.540
		2	8037		299	0.070
		3	12055		448	0.008
		4	16074		597	0.013
		5	20092		747	0.020
		6	24110		896	0.019

intermittent fashion, switching between 300 mg and 600 mg daily, would further enhance the tolerability and make use of the latent effect of lamivudine high doses on viral serum titers.

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References

1. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol. 2008; 48: 335-352.
2. McMahon BJ. Epidemiology and natural history of hepatitis B. Semin Liver Dis. 2005; 1: 3-8.
3. Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, et al. Early detection of viral resistance by determination of hepatitis B virus polymerase mutations in patients treated by lamivudine for chronic hepatitis B. Hepatology. 2000; 32:1078-1088.
4. Villeneuve JP, Durantel D, Durantel S, Westland C, Xiong S, Brosgart CL, et al. Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. J Hepatol. 2003; 39: 1085-1089.
5. Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis. 2003; 36: 687-696.
6. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004; 126: 1750-1758.
7. Zoulim F. Antiviral therapy of chronic hepatitis B: can we clear the virus and prevent drug resistance? Antivir Chem Chemother. 2004; 15: 299-305.
8. Coates JA, Cammack N, Jenkinson HJ, Jowett AJ, Jowett MI, Pearson BA, et al. (-)-2'-deoxy-3'-thiacytidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and type 2 replication in vitro. Antimicrob Agents Chemother. 1992; 36: 733-739.
9. Hart GJ, Orr DC, Penn CR, Figueiredo HT, Gray NM, Boehme RE, et al. Effects of (-)-2'-deoxy-3'-thiacytidine (3TC) 5'-triphosphate on human immunodeficiency virus reverse transcriptase and mammalian DNA polymerases alpha, beta, and gamma. Antimicrob Agents Chemother. 1992; 36: 1688-1694.
10. Doong SL, Tsai CH, Schinazi RF, Liotta DC, Cheng YC. Inhibition of the replication of hepatitis B virus in vitro by 2',3'-dideoxy-3'-thiacytidine and related analogues. Proc Natl Acad Sci USA. 1991; 88: 8495-8499.
11. Chang CN, Doong SL, Zhou JH, Beach JW, Jeong LS, Chu CK, et al. Deoxycytidine deaminase-resistant stereoisomer is the active form of (+/-)-2',3'-dideoxy-3'-thiacytidine in the inhibition of hepatitis B virus replication. J Biol Chem. 1992; 267: 13938-13942.
12. Severini A, Liu XY, Wilson JS, Tyrrell DL. Mechanism of inhibition of duck hepatitis B virus polymerase by (-)-beta-L-2',3'-dideoxy-3'-thiacytidine. Antimicrob Agents Chemother. 1995; 39: 1430-1435.
13. Boni C, Bertoletti A, Penna A, Cavalli A, Pilli M, Urbani S, et al. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J Clin Invest. 1998; 102: 968-795.
14. Boni C, Penna A, Ogg GS, Bertoletti A, Pilli M, Cavallo C, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology. 2001; 33: 963-971.
15. Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. Geneva. 2015.

16. Honkoop P, de Man RA, Niesters HG, Main J, Nevens F, Thomas HC, et al. Quantitative hepatitis B virus DNA assessment by the limiting-dilution polymerase chain reaction in chronic hepatitis B patients: evidence of continuing viral suppression with longer duration and higher dose of lamivudine therapy. Journal of viral hepatitis. 1998; 5: 307-312.

17. Musher DR, Wasser WG, Saggi SJ. High dose lamivudine and hepatitis B. The American journal of gastroenterology. 1999; 94: 3372.

18. Wani HU, Al Kaabi S, Sharma M, Singh R, John A, Derbala M, et al. High dose of Lamivudine and resistance in patients with chronic hepatitis B. Hepatitis research and treatment. 2014; 5.

19. Mason WS, Cullen J, Saputelli J, Wu TT, Liu C, London WT, et al. Characterization of the antiviral effects of 2' carbodeoxyguanosine in ducks chronically infected with duck hepatitis B virus. Hepatology. 1994; 19: 398-3411.

20. Le Guerhier F, Pichoud C, Guerret S, Chevallier M, Jamard C, Hantz O, et al. Characterization of the antiviral effect of 2',3'-dideoxy-2', 3'-didehydro-beta-L-5-fluorocytidine in the duck hepatitis B virus infection model. Antimicrob Agents Chemother. 2000; 44: 111-122.

21. Seigneres B, Martin P, Werle B, Schorr O, Jamard C, Rimsky L, et al. Effects of pyrimidine and purine analog combinations in the duck hepatitis B virus infection model. Antimicrob Agents Chemother. 2003; 47: 1842-1852.

22. Cova L, Zoulim F. Duck hepatitis B virus model in the study of hepatitis B virus. Methods Mol Med. 2004; 96: 261-268.

23. Walters KA, Joyce MA, Addison WR, Fischer KP, Tyrrell DL. Superinfection exclusion in duck hepatitis B virus infection is mediated by the large surface antigen. Journal of virology. 2004; 78: 7925-7937.

24. Johnson MA, Moore KH, Yuen GJ, Bye A, Pakes GE. Clinical pharmacokinetics of lamivudine. Clin Pharmacokinet. 1999; 36: 41-66.

25. Rajagopalan P, Boudinot FD, Chu CK, Tennant BC, Baldwin BH, Schinazi RF. Pharmacokinetics of (-)-2'-3'-dideoxy-3'-thiacytidine in woodchucks. Antimicrobial agents and chemotherapy. 1996; 40: 642-645.

26. Hussey EK, Donn KH, Daniel MJ, Hall ST, Harker AJ, Evans GL. Interspecies scaling and pharmacokinetic parameters of 3TC in humans. J Clin Pharmacol. 1994; 34: 975-977.

27. Funk A, Mhamdi M, Will H, Sirma H. Avian hepatitis B viruses: molecular and cellular biology, phylogenesis, and host tropism. World J Gastroenterol. 2007; 13: 91-103.

28. Galibert F, Chen TN, Mandart E. Localization and nucleotide sequence of the gene coding for the woodchuck hepatitis virus surface antigen: comparison with the gene coding for the human hepatitis B virus surface antigen. Proc Natl Acad Sci USA. 1981; 78: 5315-5319.

29. Millman I, Halbherr T, Simmons H. Immunological cross-reactivities of woodchuck and hepatitis B viral antigens. Infect Immun. 1982; 35:752-757.

30. Werner BG, Smolec JM, Snyder R, Summers J. Serological relationship of woodchuck hepatitis virus to human hepatitis B virus. J Virol. 1979; 32: 314-322.

31. Bullard RW, Funkhouser GE. Estimated regional blood flow by rubidium 86 distribution during arousal from hibernation. Am J Physiol. 1962; 203: 266-270.

32. Korba BE, Cote P, Hornbuckle W, Tennant BC, Gerin JL. Treatment of chronic woodchuck hepatitis virus infection in the Eastern woodchuck (Marmota monax) with nucleoside analogues is predictive of therapy for chronic hepatitis B virus infection in humans. Hepatology. 2000; 31: 1165-1175.

33. Michalak TI, Zhang H, Churchill ND, Larsson T, Johansson NG, Oberg B. Profound antiviral effect of oral administration of MIV-210 on chronic hepadnaviral infection in a woodchuck model of hepatitis B. Antimicrobial agents and chemotherapy. 2009; 53: 3803-3814.

34. Addison WR, Walters KA, Wong WW, Wilson JS, Madej D, Jewell LD, et al. Half-life of the duck hepatitis B virus covalently closed circular DNA pool in vivo following inhibition of viral replication. Journal of virology. 2002; 76: 6356-6363.

35. Walters KA, Tipples GA, Allen MI, Condreay LD, Addison WR, Tyrrell L. Generation of stable cell lines expressing Lamivudine-resistant hepatitis B virus for antiviral-compound screening. Antimicrobial agents and chemotherapy. 2003; 47: 1936-1942.

36. Delmas J, Schorr O, Jamard C, Gibbs C, Trepo C, Hantz O, et al. Inhibitory effect of adefovir on viral DNA synthesis and covalently closed circular DNA formation in duck hepatitis B virus-infected hepatocytes in vivo and in vitro. Antimicrobial agents and chemotherapy. 2002; 46: 425-433.

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