Characterization of HBV kinetics during infection and treatment in primary human hepatocytes

Y. Ishida[1,2*], P. Dubey[3*], M. Tsuge[2,3,4], M. Yamao[1], C. Tateno[1,2], S. L. Uprichard[3], H. Dahari[3#], K. Chayama[2, 4#]

(1) PhoenixBio Co., Ltd., Higashi-Hiroshima, Japan; (2) Research Center for Hepatology and Gastroenterology, Hiroshima University, Hiroshima, Japan; (3) The Program for Experimental & Theoretical Modeling, Division of Hepatology, Department of Medicine, Loyola University Medical Center, Maywood, IL, USA; (4) Department of Gastroenterology and Metabolism, Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan.
*These authors contributed equally to this study; #corresponding authors

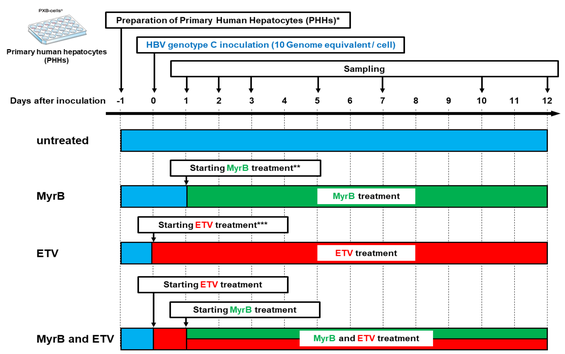
INTRODUCTION

- Chronic hepatitis B virus (HBV) infection is a serious global health problem, which causes advanced liver diseases, such as cirrhosis and hepatocellular carcinoma.
- Understating of early HBV dynamics at the molecular level in primary human hepatocytes (PHHs) is lacking.

AIM

- To provide detailed characterization of HBV kinetics during infection and treatment in primary human hepatocytes .

MATERIAL & METHODS



* PHHs were isolated from human hepatocyte chimeric mice (PXB mouse, Phoenix Bio Co, Ltd, Hiroshima, Japan) and 2.1×10^5 cells/cm^2 were seeded on 24-well plates.
** MyrB treatment (6.25 μg/mL) was started from day 1 post inoculation.
*** ETV treatment (10 μM) was started at same time point of the inoculation.

Fig 1. Experiment Design

Quantification of HBV DNA and HBsAg in culture media

- Intracellular and extracellular HBV DNA levels and covalently closed circular DNA (cccDNA) levels were determined by quantitative real-time PCR (qPCR).
- For intracellular HBV DNA and cccDNA levels, qPCR was performed using 100ng of DNA.
- HBsAg was quantified by enzyme-linked immunosorbent assay.

Estimation of HBV infected cells at day 12

- At 12 days post-inoculation, PHHs were fixed with 10% formalin and stained with anti-HBsAg antibody (Thermo Fisher Scientific, Rockford, IL).
- To analyze the percent HBsAg-positive PHHs, five pictures were taken with BZ-X700 microscope (Keyence, Osaka, Japan) and the number of PHHs and HBsAg-positive PHHs were counted.

RESULTS

Extracellular & Intracellular HBV DNA Fig. 2 A & B

Four phases in untreated PHHs

- **Phase 1 –** 3 day decline of half-life, $t_{1/2}$=9 h and 2 day decline of $t_{1/2}$=24 h in extracellular and intracellular HBV DNA, respectively
- **Phase 2 -** 2-3 day eclipse (or plateau) in extracellular and intracellular HBV DNA, respectively
- **Phase 3** – Rapid increased (doubling time, t_2=17h and t_2=24 h), in extra- and intracellular DNA, respectively
- **Phase 4** - Slower increase (t_2=56 h and 77 h) from day 7 to end of experiment, respectively

Myrcludex-B (MyrB) had no effect

Entecavir (ETV) suppressed production

- A similar 1st phase decline was observed, but no subsequent increase.

Extracellular HBsAg Fig. 2C

Two phases in untreated PHHs

- **Phase 1** – 3 day rapid decline with slope of ~1.08 log/day ($t_{1/2}$=7 hr)
- **Phase 2** – Increase (t_2=47 hr) from day 3 p.i until end of experiment

Myrcludex-B (MyrB) had no effect

Entecavir (ETV) suppressed production

- A similar 1st phase decline was observed, but no subsequent increase.

HBV cccDNA Fig. 2D

Three phases in untreated PHHs

- **Phase 1** – 2 day rapid increase with slope of ~ 1 log/day (t_2=7 hr)
- **Phase 2** - Plateau from day 2-5 pi
- **Phase 3** - Slow increase (t_2=98 hr) from day 5 to end of experiment

Myrcludex-B (MyrB) prevented Phase 3 increase

- Under MyrB cccDNA remains in plateau phase (t_2=672 hr) from day 2-12 pi perhaps reflecting inhibition of spread.

Under Entecavir (ETV), cccDNA showed a slower, biphasic amplification

- **Phase 1** was prolonged until day 5 p.i. (t_2=31 hr) (significantly slower than untreated PHHs, P=0.024) but consisted of 2 distinct amplification waves. Regardless, at day 5 pi, cccDNA reached to the similar level as untreated PHHs.
- **Phase 2** - After day 5 p.i. cccDNA remained at a plateau.

Kinetic analysis of HBV post inoculation in PHHs

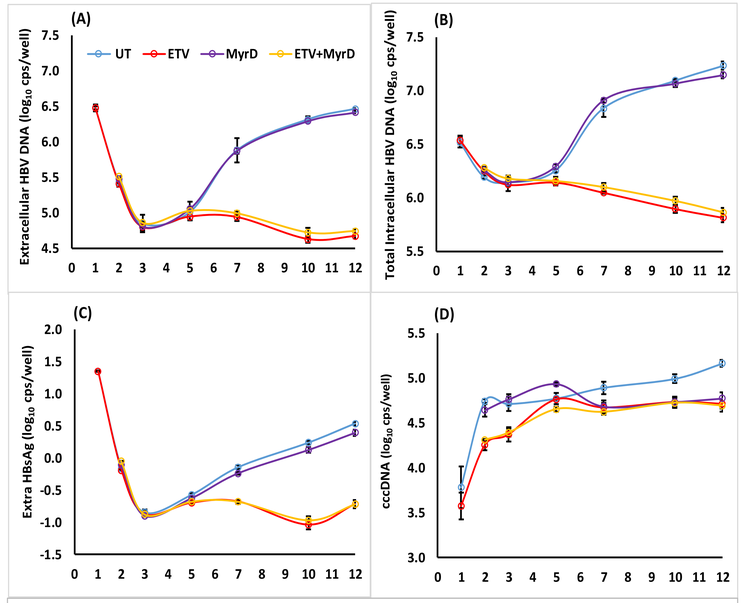


Fig 2. HBV kinetics during infection and treatment. (A) extracellular HBV DNA, **(B)** total intracellular HBV DNA, **(C)** HBsAg and **(D)** cccDNA, during Mock treatment (UT) or treatment with entecavir (ETV), myrcludex (MyrB) or ETV+MyrB. Black vertical error bars represent standard deviation.

CONCLUSION

- HBV infection kinetics in PHHs is multiphasic
- When initiated at the time of inoculation, ETV does not prevent infection, but does prevent intracellular and extracellular HBV DNA amplification, HBsAg secretion, and slows the accumulation of cccDNA
- As expected, MyrB treatment 24 h post-inoculation did not prevent infection, but the lack of effect on subsequent intracellular and extracellular HBV DNA and HBsAg kinetics indicates insignificant HBV spread during this 12 day experiment.

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CONTACT INFORMATION

E-mail: chayama@hiroshima-u.ac.jp

E-mail: hdahari@luc.edu