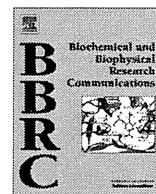
Biochemical and Biophysical Research Communications 443 (2014) 808–813

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc



Evaluation and identification of hepatitis B virus entry inhibitors using HepG2 cells overexpressing a membrane transporter NTCP ☆

Masashi Iwamoto [a,b], Koichi Watashi [a,b,*], Senko Tsukuda [a,c], Hussein Hassan Aly [a], Masayoshi Fukasawa [d], Akira Fujimoto [a], Ryosuke Suzuki [a], Hideki Aizaki [a], Takayoshi Ito [e], Osamu Koiwai [b], Hiroyuki Kusuhara [f], Takaji Wakita [a]

[a] Department of Virology II, National Institute of Infectious Diseases, Tokyo 162-8640, Japan
[b] Department of Applied Biological Science, Tokyo University of Sciences, Noda 278-8510, Japan
[c] Micro-signaling Regulation Technology Unit, RIKEN Center for Life Science Technologies, Wako 351-0198, Japan
[d] Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan
[e] Division of Gastroenterology, Department of Medicine, Showa University School of Medicine, Tokyo 142-8666, Japan
[f] The University of Tokyo, Graduate School of Pharmaceutical Sciences, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 24 November 2013
Available online 14 December 2013

Keywords:
HBV
Infection
NTCP
DMSO
Cyclosporin
Oxysterol

ABSTRACT

Hepatitis B virus (HBV) entry has been analyzed using infection-susceptible cells, including primary human hepatocytes, primary tupaia hepatocytes, and HepaRG cells. Recently, the sodium taurocholate cotransporting polypeptide (NTCP) membrane transporter was reported as an HBV entry receptor. In this study, we established a strain of HepG2 cells engineered to overexpress the human NTCP gene (HepG2-hNTCP-C4 cells). HepG2-hNTCP-C4 cells were shown to be susceptible to infection by blood-borne and cell culture-derived HBV. HBV infection was facilitated by pretreating cells with 3% dimethyl sulfoxide permitting nearly 50% of the cells to be infected with HBV. Knockdown analysis suggested that HBV infection of HepG2-hNTCP-C4 cells was mediated by NTCP. HBV infection was blocked by an anti-HBV surface protein neutralizing antibody, by compounds known to inhibit NTCP transporter activity, and by cyclosporin A and its derivatives. The infection assay suggested that cyclosporin B was a more potent inhibitor of HBV entry than was cyclosporin A. Further chemical screening identified oxysterols, oxidized derivatives of cholesterol, as inhibitors of HBV infection. Thus, the HepG2-hNTCP-C4 cell line established in this study is a useful tool for the identification of inhibitors of HBV infection as well as for the analysis of the molecular mechanisms of HBV infection.

© 2013 The Authors. Published by Elsevier Inc. All rights reserved.

1. Introduction

Approximately 350 million people are estimated to be infected with hepatitis B virus (HBV) worldwide [1–4]. Chronically infected patients are at a greater risk of developing hepatocellular carcinoma. Currently, clinical treatment for HBV infection includes interferon (IFN)α and nucleos(t)ide analogs [2,4]. IFNα therapy yields long-term clinical benefit in less than 40% of the treated patients and can cause significant side effects. Nucleos(t)ide analog treatment can suppress HBV replication with substantial biochemical and histological improvement; however, such analogs may select drug-resistant viruses, thereby limiting the efficacy of long-term treatment. Thus, the development of new anti-HBV agents targeting a different molecule in the HBV life cycle is urgently needed.

HBV is a hepatotropic virus that mainly or exclusively infects human liver [1,5]. HBV infection can be reproduced in cell culture using primary human hepatocytes (PHH), primary tupaia hepatocytes (PTH), and HepaRG cells [6]. Although HBV infection into these cells is robust, these models have significant limitations as tools for analyzing the mechanisms of HBV infection. Notably, these models can yield unstable reproducibility among lots and low tolerability of transfection efficiency with plasmid and siRNA: preparation and culturing of these cells require significant

Abbreviations: Ab, antibody; cccDNA, covalently closed circular DNA; Cs, cyclosporin; DMSO, dimethyl sulfoxide; GEq, genome equivalent; HBc, HBV core protein; HBs, HBV surface protein; HBV, hepatitis B virus; NTCP, sodium taurocholate cotransporting polypeptide; OHC, hydroxycholesterol; PHH, primary human hepatocytes; PTH, primary tupaia hepatocytes.

☆ This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

* Corresponding author at: Department of Virology II, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. Fax: +81 3 5285 1161.
E-mail address: kwatashi@nih.go.jp (K. Watashi).

0006-291X/$ - see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.12.052

technical skill. In the case of hepatitis C virus (HCV), development of the HCV cell culture (HCVcc) system, in which HCV produced from a JFH-1 strain-based molecular clone can reinfect Huh-7 cells, greatly contribute to the characterization of the HCV life cycle and the evaluation of novel anti-HCV drug candidates [7]. However, the above-noted limitations of HBV-susceptible cells have hampered analysis of the HBV life cycle and impeded identification of new anti-HBV drug targets. Thus, establishment of a novel cell line supporting HBV infection is expected to accelerate the molecular analyses of HBV infection as well as the development of anti-HBV agents.

Recently, the sodium taurocholate cotransporting polypeptide (NTCP) membrane transporter was reported as an HBV entry receptor [8]. NTCP is a sodium-dependent transporter for taurocholic acid, and belongs to a family of solute carrier proteins that consist of seven members (SLC10A1-A7) [9,10]. NTCP is expressed at the basolateral membrane of hepatocytes and mediates the transport of conjugated bile acids and some drugs from portal blood to the liver [11]. NTCP specifically interacts with the large surface protein of HBV, thereby functioning as a viral entry receptor [8].

In this study, we established a strain of HepG2 cells engineered to overexpress the NTCP-encoding gene. One of these clones, designated HepG2-hNTCP-C4, was shown to be highly susceptible for HBV infection, confirming that this infection is mediated by NTCP and permitting evaluation in these cells of the anti-HBV activity of various compounds: reduction of HBV infection of HepG2-hNTCP-C4 cells was observed upon treatment with compounds that blocked HBV entry in other assays and by known inhibitors of NTCP transporter activity [12]. A small-scale chemical screen permitted use to identify oxysterols as inhibitors of HBV infection. Thus, the cell line established in this study is useful for screening for anti-HBV agents, as well as for analysis of the molecular mechanisms of HBV infection.

2. Materials and methods

2.1. Reagents

Dimethyl sulfoxide (DMSO), anti-FLAG antibody (Ab), dextran sulfate, cholate, progesterone, 22(S)-hydroxycholesterol (OHC), 25-OHC, 20α-OHC, and 7β-OHC were purchased from Sigma. Ursodeoxycholate was purchased from Tokyo Chemical Industry. Bromosulfophthalein was from MP biomedicals. Cyclosporin (Cs)A, CsB, CsC, CsD, and CsH were obtained from Enzo Lifesciences. Anti-HBV surface protein (HBs) Ab was from Abcam. Heparin was obtained from Mochida Pharmaceuticals. Myrcludex-B was kindly provided by Dr. Stephan Urban at University Hospital Heidelberg and was synthesized by CS Bio (Shanghai, China).

2.2. Cell culture and plasmid transfection

HepG2 and HepG2-hNTCP-C4 cells were cultured with DMEM/F-12 + GlutaMax (Invitrogen) supplemented with 10 mM HEPES (Invitrogen), 200 units/ml penicillin, 200 μg/ml streptomycin, 10% FBS, 50 μM hydrocortisone and 5 μg/ml insulin in the presence (HepG2-hNTCP-C4 cells) or absence (HepG2 cells) of 400 μg/ml G418 (Nacalai). HepAD38 (kindly provided by Dr. Christoph Seeger at Fox Chase Cancer Center) [13] and HepaRG cells (BIOPREDIC) were cultured as described previously [14].

An expression plasmid for hNTCP [15] was transfected into HepG2 cells with TransIT-LT1 (Mirus) according to the manufacturer's instruction to establish HepG2-hNTCP-C4 cells.

2.3. HBV preparation and infection

HBV was prepared and infected as described [14]. Except as noted, the HBV used in this study was genotype D derived from HepAD38 cells [13]. HBV was infected into NTCP-expressing HepG2 cells at 6×10^3 or 1.8×10^4 genome equivalent (GEq)/cell or into HepaRG cells at 6×10^3 GEq/cell. All infections were performed in the presence of 4% PEG8000 at 37 °C for 16 h as previously described [14]. Dr. Urban's group reported that a quantity of more than 10^4 GEq/cell (i.e. $1.25 - 40 \times 10^4$ GEq/cell) of HBV derived from HepAD38 or HepG2.2.15 cells was required as an inoculum for efficient infection into HepaRG cells in the presence of 4% PEG8000 [16]. A limited number of infections were performed with HBV of genotype C, derived from the serum of an HBV-infected patient, at 100 GEq/cell.

2.4. Real-time PCR and RT-PCR

Real-time PCR for quantification of HBV covalently closed circular (ccc)DNA were performed as described [14]. Isolation of total RNA from cell lysates and reverse transcription PCR (RT-PCR) using a One step RNA PCR kit (Takara) were performed as described previously [17]. Primers used in this study were as follows: 5′-AGGGAGGAGGTGGCAATCAAGAGTGG-3′ and 5′-CCGGCTGAAGAACATTGAGGCACTGG-3′ for NTCP, 5′-CCATGGAGAAGGCTGGGG-3′ and 5′-CAAAGTTGTCATGGATGACC-3′ for GAPDH, respectively.

2.5. Detection of HBs and HBe antigens

HBs antigen was quantified by ELISA as described previously [14]. HBe antigen was detected by Chemiluminescent Immuno Assay (Mitsubishi Chemical Medience).

2.6. Southern blot analysis

Isolation of cellular DNA and southern blot analysis to detect HBV DNAs were performed as described previously [14].

2.7. Indirect immunofluorescence analysis

Immunofluorescence was conducted essentially as described [14] using an anti-HBc Ab (#B0586, DAKO) at a dilution of 1:1000.

2.8. Flow cytometry

An aliquot of 1×10^6 of HepG2 or HepG2-hNTCP-C4 cells was incubated for 30 min with a 1:50 dilution of anti-NTCP Ab (Abcam), then washed and incubated with a dye-labeled secondary Ab (Alexa Fluor 488, Invitrogen) at 1:500 dilution in the dark. Staining and washing were carried out at 4 °C in PBS supplemented with 0.5% bovine serum albumin and 0.1% sodium azide. The signals were analyzed with Cell Sorter SH8000 (SONY).

2.9. siRNA transfection

siRNAs were transfected into the cells at a final concentration of 10–30 nM using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol. siRNAs were purchased from Sigma.

2.10. Statistical analyses

Statistical analyses are done with student *t*-test.

3. Results and discussion

3.1. Establishment of a cell line susceptible to HBV infection

To establish a cell line permanently expressing NTCP, we transfected an NTCP-encoding plasmid into HepG2 cells and selected with G418 at 1 mg/ml for 3 weeks. The resultant 9 cell clones were isolated and NTCP expression was analyzed by RT-PCR. One of these clones, designated HepG2-hNTCP-C4, was used in the following experiments because this specific clone exhibited high expression of NTCP and high susceptibility to HBV infection, as shown below. Specifically, NTCP mRNA was abundantly expressed in HepG2-hNTCP-C4 cells, in contrast to little to no expression of NTCP mRNA in the parental HepG2 cells (Fig. 1A). Consistent with the mRNA levels, NTCP protein was detected on the cell surface in HepG2-hNTCP-C4 cells (Fig. 1B). To evaluate HBV infection, these cells were inoculated with HBV for 16 h and cultured in normal growth medium for an additional 12 days, and then HBV surface protein (HBs) and HBe antigens in the culture supernatant as well as HBV DNAs, covalently closed circular (ccc)DNA, and HBV core (HBc) in the cells were assessed. The HBV inoculum used in this experiment was of genotype D, and was derived from the culture supernatant of HepAD38 cells that produce HBV by depletion of tetracycline [13]. To confirm that the detected signals were derived from HBV infection and did not represent non-specific background, the cells were incubated with 1 μM Myrcludex-B (or with DMSO vehicle) for 3 h prior to and for 16 h during HBV infection. Myrcludex-B is a lipopeptide consisting of amino acid residues 2–48 of the pre-S1 region of HBV, and is known to block HBV entry [18]. Following HBV exposure, little or no HBs and HBe antigens was detected in the culture supernatant of the parental HepG2 cells, and little HBc protein was observed in these cells (Fig. 1C, D, and G). However, these proteins, as well as HBV DNAs and cccDNA, were detected in HBV-treated HepG2-hNTCP-C4 cells (Fig. 1C–G). The corresponding signals were significantly reduced in the cells treated with an HBV entry inhibitor, Myrcludex-B, but not in the cells treated with DMSO (Fig. 1C–G). These data suggested that HepG2-hNTCP-C4 cells are HBV-susceptible, in contrast to the parental HepG2 cells. The HepG2-hNTCP-C4 cell line also was susceptible to infection with HBV genotype C, which was derived from the serum of an HBV-infected patient (Fig. 1H and I).

3.2. HBV susceptibility of HepG2-hNTCP-C4 cells was augmented by pretreatment with DMSO

It has been reported that a prolonged HBV infection in primary human hepatocytes can be enhanced by pretreatment with DMSO [19]. Therefore, we examined whether pretreatment with DMSO affected HBV infection of HepG2-NTCP-C4 cells. The cells were pretreated with 3% DMSO for 24 h and then the HBV infectivity was investigated following the protocol as in Fig. 1. Immunofluorescence analysis revealed that approximately 50% of the DMSO-pretreated cells were HBc-positive at 12 days post-infection (Fig. 2A, middle), while only 10–20% of cells were HBc-positive cells in the absence of pretreatment (Fig. 1G, upper right). The effect of DMSO pretreatment on HBV susceptibility was both concentration-(Fig. 2B) and time-dependent (Fig. 2C).

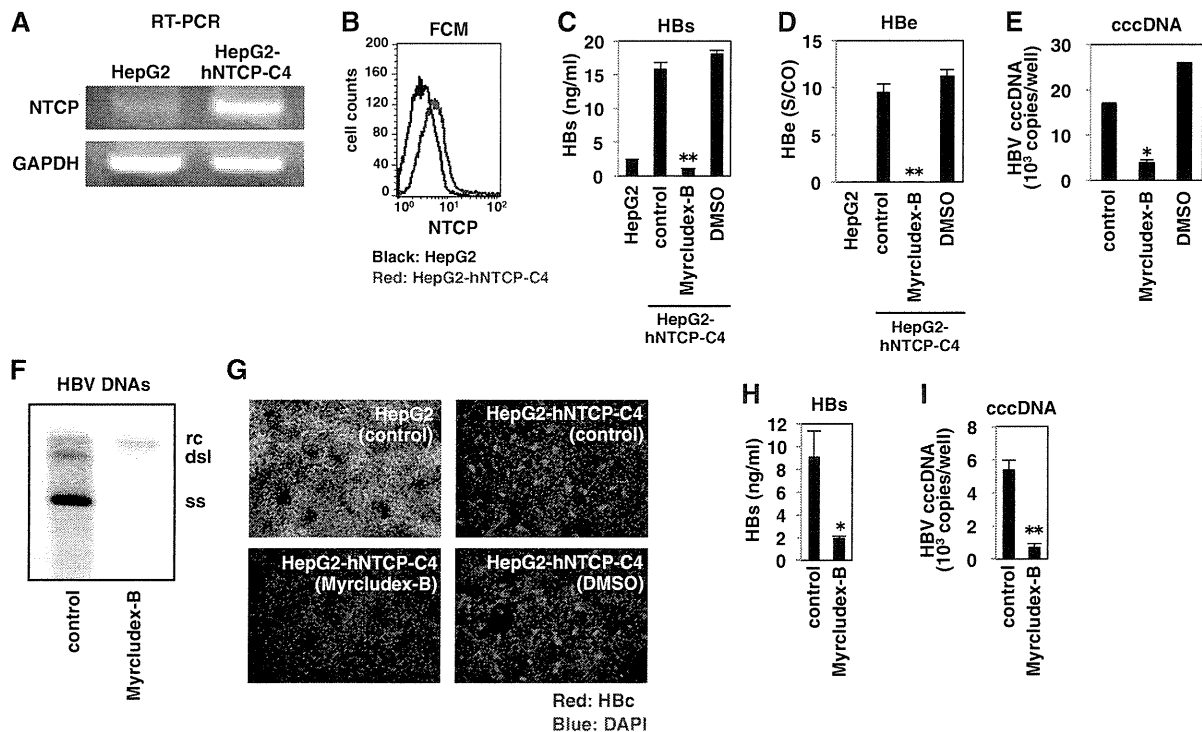


Fig. 1. Establishment of a cell line susceptible to hepatitis B virus (HBV) infection. (A) mRNAs for sodium taurocholate cotransporting polypeptide (NTCP) and GAPDH in HepG2 and HepG2-hNTCP-C4 cells were detected by RT-PCR. (B) NTCP protein on cell surface of HepG2 (black) and HepG2-hNTCP-C4 cells (red) was detected by flow cytometry. (C-G) HepG2-hNTCP-C4 or the parental HepG2 cells pretreated with or without 1 μM Myrcludex-B or vehicle (DMSO) for 3 h were inoculated with HBV (genotype D) for 16 h. After washing out of the free virus and the compounds, the cells were cultured for an additional 12 days in normal growth medium and then assayed for secretion of HBs (C) and HBe antigens (D) secreted in the culture supernatant, and for the presence of HBV covalently closed circular (ccc)DNA (E), HBV DNAs (F), and HBV core (HBc) proteins (G) in the cells. rc, dsl, and ss in (F) indicate relaxed circular, double strand linear, and single strand HBV DNA, respectively. Red and blue signals in (G) indicate HBc protein and nuclear staining, respectively. (H and I) Infection of blood-borne HBV into HepG2-hNTCP-C4 cells. HBV (genotype C) derived from an HBV-infected patient was used as an inoculum for the infection assay. Levels for HBs antigen in the culture supernatant (H) and HBV cccDNA in the cells (I) are shown. The data in C–E, H, and I show the means of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

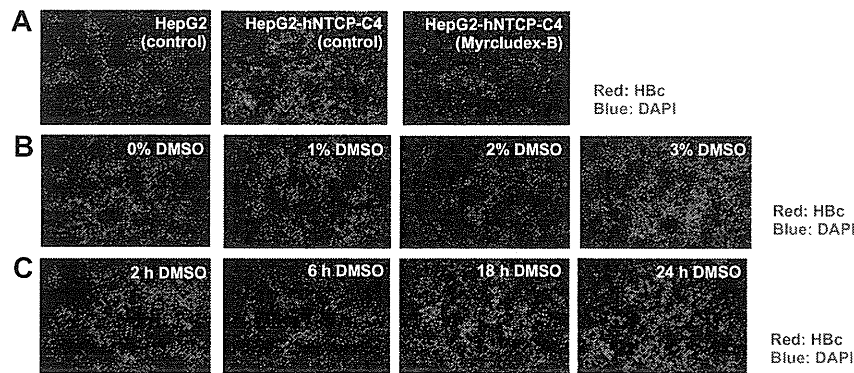


Fig. 2. HBV infection was facilitated by pretreatment of HepG2-hNTCP-C4 cells with DMSO. (A) HepG2 or HepG2-hNTCP-C4 cells preincubated with 3% DMSO for 24 h were inoculated with HBV in the presence of 3% DMSO for 16 h. Treatment with Myrcludex-B was used as a negative control for infection. At 12 days postinfection, HBc protein (red) and the nucleus (blue) were detected by immunofluorescence analysis. (B) Cells were pretreated by exposure for 24 h to various concentrations of DMSO (0–3%). (C) Cells were pretreated by exposure to 3% DMSO for various treatment times (2, 6, 18, and 24 h). HBc protein (red) and the nucleus (blue) were detected as in (A).

3.3. HBV infection was mediated by NTCP in HepG2-hNTCP-C4 cells

We used knockdown analysis to determine whether HBV infection of HepG2-hNTCP-C4 cells was mediated by NTCP. Transfection with siRNA against NTCP (si-NTCP) and GAPDH (si-GAPDH) specifically knocked down mRNA for NTCP and GAPDH, respectively, in HepG2-hNTCP-C4 cells (Fig. 3A). Consistent with the effect on transcript level, treatment with si-NTCP depleted NTCP protein on the cell surface (Fig. 3B). The HBV infection assay, performed as in Fig. 1, indicated that depletion of NTCP reduced the levels for HBs (Fig. 3C) and HBe antigens (Fig. 3D) in culture supernatant as well as HBV cccDNA (Fig. 3E) and HBc protein (Fig. 3F) in the cells at 12 days postinfection with HBV. These data suggested that HBV infection into HepG2-hNTCP-C4 cells was mediated by NTCP.

3.4. Evaluation of HBV entry inhibitors in HepG2-hNTCP-C4 cells

To determine whether HepG2-hNTCP-C4 cells could be used to evaluate anti-HBV activity of compounds, we examined the effect of known entry inhibitors in these cells. The cells were pretreated with compounds for 3 h and then inoculated with HBV for 16 h in the presence of compounds (Fig. 4A). Inoculation with HBV was followed by culturing of the cells in normal growth medium for an additional 12 days until detection of HBs antigen in the culture supernatant and cccDNA in the cells (Fig. 4A). This protocol has been used previously to evaluate the entry inhibition activity of compounds [20]. Treatment with anti-HBs neutralizing Ab, but not that with a non-relevant anti-FLAG Ab, inhibited HBV infection (Fig. 4B). Heparin and dextran sulfate, which have been reported to inhibit HBV attachment to the target cells [21], also reduced HBV infection (Fig. 4C). In addition, known NTCP substrates and inhibitors, including ursodeoxycholate, cholate, progesterone, and bromosulfophthalein [12], blocked HBV infection in this assay (Fig. 4D). We recently identified that cyclosporin A (CsA) and its analogs blocked HBV entry through inhibition of interaction between NTCP and the HBV large surface protein [20]. As shown in Fig. 4E, CsA and its analogs inhibited HBV infection in the present assay, with CsB showing the highest potency for inhibition of HBV infection among Cs analogs (Fig. 4E). These data indicate that HepG2-hNTCP-C4 cells are useful for evaluating the effect of HBV entry inhibitors.

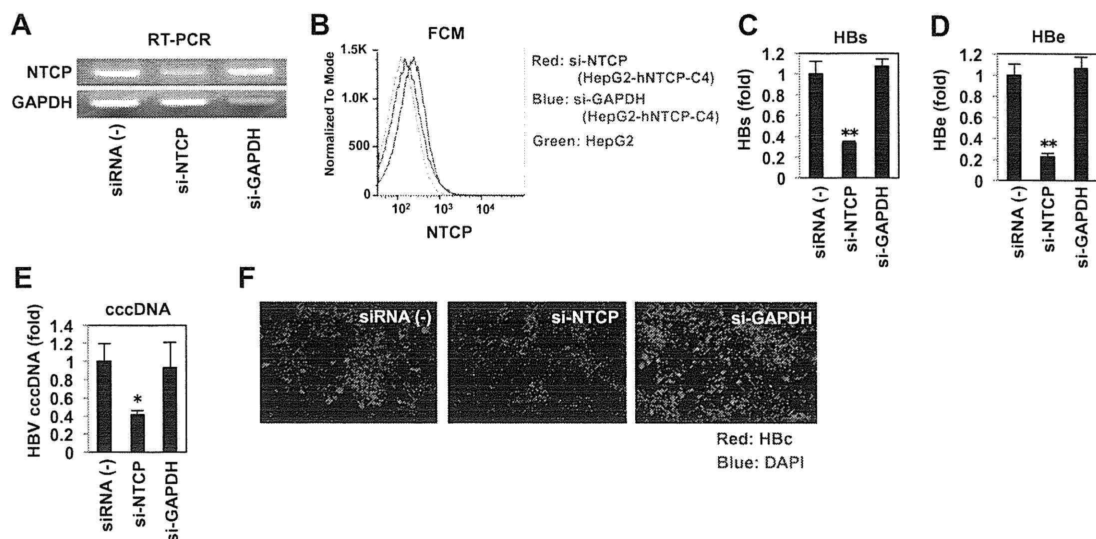


Fig. 3. HBV infection of HepG2-hNTCP-C4 cells was mediated by NTCP. (A) HepG2-hNTCP-C4 cells were transfected (for 48 h) with or without [siRNA(−)] siRNAs against NTCP (si-NTCP) or GAPDH (si-GAPDH), and mRNA expression levels of NTCP and GAPDH were detected by RT-PCR. (B) Parental HepG2 and HepG2-hNTCP-C4 cells were transfected (for 48 h) with or without si-NTCP or si-GAPDH, and cell surface-displayed NTCP protein was detected by flow cytometry. The red, blue, and green lines indicate the signal in HepG2-hNTCP-C4 cells treated with si-NTCP, HepG2-hNTCP-C4 cells treated with si-GAPDH, and HepG2 cells, respectively. (C–F) The cells prepared as in (A) were infected with HBV according to the protocol shown in Fig. 1. Culture supernatants were assayed for levels of secreted HBs (C) and HBe (D) antigens, and cells were assayed for intracellular levels of HBV cccDNA (E) and HBc protein (F). The red and blue signals in (F) indicate HBc and nuclear staining, respectively.

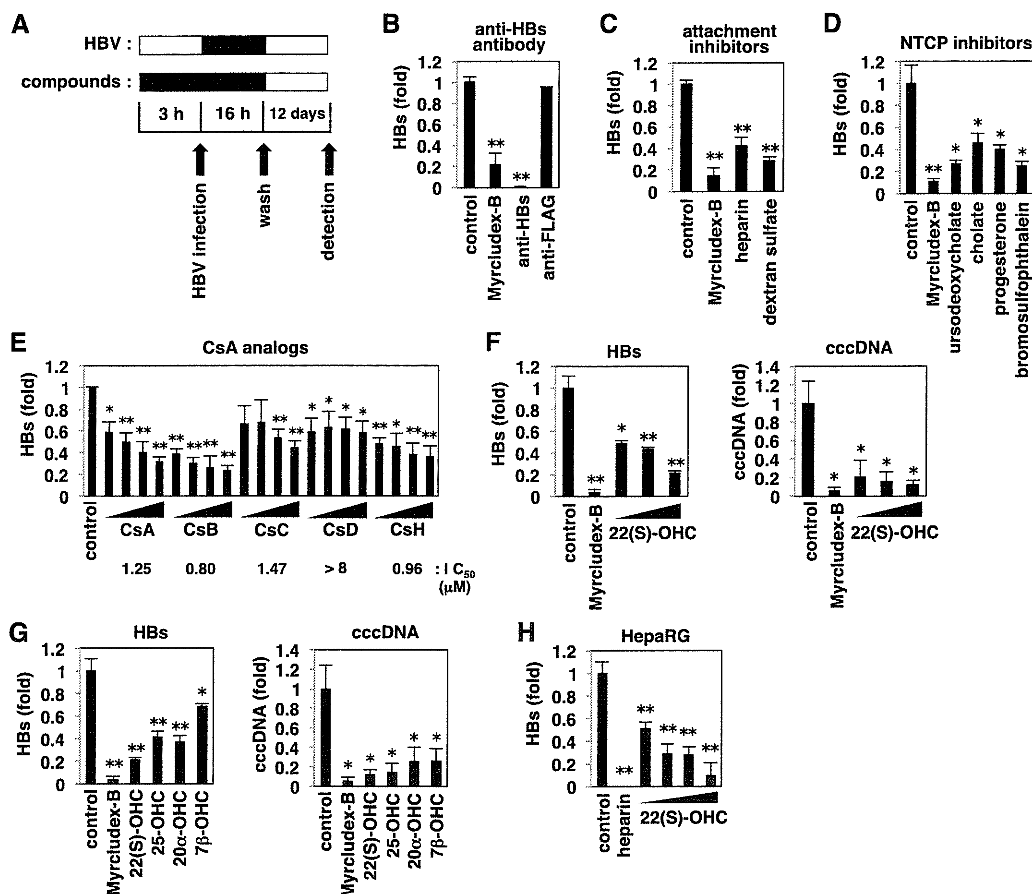


Fig. 4. Evaluation of HBV entry inhibitors in HepG2-hNTCP-C4 cells. (A) Schematic representation of the experimental procedure for evaluating HBV entry inhibition. HepG2-hNTCP-C4 cells were pretreated with or without compounds for 3 h and then inoculated with HBV for 16 h. After washing out of free HBV and the compounds, the cells were cultured with normal culture medium in the absence of compounds for an additional 12 days, and HBs antigen in the culture supernatant and/or HBV cccDNA in the cells were detected. Black and white bars show period of treatment and without treatment, respectively. (B–G) HepG2-hNTCP-C4 cells were treated with or without 1 μM Myrcludex-B, 10 μg/ml anti-HBs or anti-FLAG Ab (B); HBV attachment inhibitors including 100 IU/ml heparin and 1 mg/ml dextran sulfate (C); NTCP inhibitors including 100 μM ursodeoxycholate, 100 μM cholate, 40 μM progesterone, and 100 μM bromosulfophthalein (D); cyclosporins (CsA, CsB, CsC, CsD, CsH) at 1, 2, 4, and 8 μM (E); 22(S)-hydroxycholesterol (OHC) at 11, 33, and 100 μM (F); or oxysterols including 22(S)-OHC, 25-OHC, 20α-OHC, and 7β-OHC at 100 μM (G). For each assay, the cells were infected with HBV as shown in (A) and the levels of HBs antigen secreted into the culture supernatant and/or cccDNA in the cells were detected. Pretreatment time of compounds in (F) and (G) was 6 h, instead of 3 h. IC_{50}s of cyclosporin derivatives calculated in this assay are shown below the graph in (E). (H) HepaRG cells were treated with or without various concentrations of 22(S)-OHC (0.3, 0.9, 3, and 9 μM) and infected with HBV according to the protocol shown in (A). HBV infection was monitored by detecting the level of HBs secreted into the culture supernatant.

As there are only reverse transcriptase inhibitors currently available as anti-HBV drugs that inhibit the HBV life cycle, development of new anti-HBV agents targeting different steps in the HBV life cycle are greatly needed [1–4]. We therefore screened for compounds that blocked HBV entry by following the same protocol as in Fig. 4A. We found that an oxysterol, 22(S)-hydroxycholesterol (OHC), reduced HBV infection in a dose-dependent manner (Fig. 4F). Other oxysterols, 25-OHC, 20α-OHC, and 7β-OHC, also significantly decreased HBV infection (Fig. 4G). To validate this result, we repeated the assay using HepaRG cells, a line that frequently has been used in HBV entry experiments [14]. We found that 22(S)-OHC also reduced HBV infection of HepaRG cells in a dose-dependent manner (Fig. 4H), suggesting that the observed inhibitory effect of oxysterols reflects a genuine inhibition of HBV infection.

Thus, we have newly established a cell line that is susceptible to HBV infection. HepG2-hNTCP-C4 cells exhibited approximately 50% of HBV-infection positive cells (Fig. 2A), while maximum HBV infection of HepaRG cells was reported to be only 7% [16] or 20% [22] of the total population. These cells are expected to be useful for analyzing the molecular mechanisms of HBV infection, given that HepG2-derived cells show higher efficiency of transfection with expression plasmids and siRNAs than the current available HBV-susceptible PHH, PTH, and HepaRG cells. HepG2-hNTCP-C4 cells will facilitate knockdown analysis of host factors to define their roles in infection and screenings of compounds to identify novel inhibitors of HBV infection. As an example, we demonstrated here that oxysterols blocked HBV infection. The molecular mechanisms whereby oxysterols inhibit HBV infection are now under investigation. These analyses will be important for understanding the mechanisms of HBV infection as well as for developing new anti-HBV agents.

Acknowledgments

HepAD38 cells were kindly provided by Dr. Christoph Seeger at Fox Chase Cancer Center. Myrcludex-B, a pre-S1 lipopeptide, was kindly provided by Dr. Stephan Urban at University Hospital Heidelberg. We also are grateful to all of the members of Department of Virology II, National Institute of Infectious Diseases. This study was supported by Grants-in-aid from the Ministry of Health, Labor, and Welfare, Japan, from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and from Japan Society for the Promotion of Science, and from the Research on Health Sciences Focusing on Drug Innovation from the Japan Health Sciences Foundation.

References

[1] D. Grimm, R. Thimme, H.E. Blum, HBV life cycle and novel drug targets, Hepatol. Int. 5 (2011) 644–653.
[2] J.M. Pawlotsky, G. Dusheiko, A. Hatzakis, D. Lau, G. Lau, T.J. Liang, S. Locarnini, P. Martin, D.D. Richman, F. Zoulim, Virologic monitoring of hepatitis B virus therapy in clinical trials and practice: recommendations for a standardized approach, Gastroenterology 134 (2008) 405–415.
[3] M. Rapicetta, C. Ferrari, M. Levrero, Viral determinants and host immune responses in the pathogenesis of HBV infection, J. Med. Virol. 67 (2002) 454–457.
[4] F. Zoulim, Hepatitis B virus resistance to antiviral drugs: where are we going?, Liver Int 31 (Suppl. 1) (2011) 111–116.
[5] P. Gripon, S. Rumin, S. Urban, J. Le Seyec, D. Glaise, I. Cannie, C. Guyomard, J. Lucas, C. Trepo, C. Guguen-Guillouzo, Infection of a human hepatoma cell line by hepatitis B virus, Proc. Natl. Acad. Sci. USA 99 (2002) 15655–15660.
[6] D. Glebe, S. Urban, Viral and cellular determinants involved in hepadnaviral entry, World J. Gastroenterol. 13 (2007) 22–38.
[7] J.M. Gottwein, J. Bukh, Cutting the gordian knot-development and biological relevance of hepatitis C virus cell culture systems, Adv. Virus Res. 71 (2008) 51–133.
[8] H. Yan, G. Zhong, G. Xu, W. He, Z. Jing, Z. Gao, Y. Huang, Y. Qi, B. Peng, H. Wang, L. Fu, M. Song, P. Chen, W. Gao, B. Ren, Y. Sun, T. Cai, X. Feng, J. Sui, W. Li, Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus, Elife 1 (2012) e00049.
[9] M.S. Anwer, B. Stieger, Sodium-dependent bile salt transporters of the SLC10A transporter family: more than solute transporters, Pflugers Arch. (2013) (Epub ahead of print).
[10] P.J. Meier, B. Stieger, Bile salt transporters, Annu. Rev. Physiol. 64 (2002) 635–661.
[11] C. Seeger, W.S. Mason, Sodium-dependent taurocholic cotransporting polypeptide: a candidate receptor for human hepatitis B virus, Gut 62 (2013) 1093–1095.
[12] R.B. Kim, B. Leake, M. Cvetkovic, M.M. Roden, J. Nadeau, A. Walubo, G.R. Wilkinson, Modulation by drugs of human hepatic sodium-dependent bile acid transporter (sodium taurocholate cotransporting polypeptide) activity, J. Pharmacol. Exp. Ther. 291 (1999) 1204–1209.
[13] S.K. Ladner, M.J. Otto, C.S. Barker, K. Zaifert, G.H. Wang, J.T. Guo, C. Seeger, R.W. King, Inducible expression of human hepatitis B virus (HBV) in stably transfected hepatoblastoma cells: a novel system for screening potential inhibitors of HBV replication, Antimicrob. Agents Chemother. 41 (1997) 1715–1720.
[14] K. Watashi, G. Liang, M. Iwamoto, H. Marusawa, N. Uchida, T. Daito, K. Kitamura, M. Muramatsu, H. Ohashi, T. Kiyohara, R. Suzuki, J. Li, S. Tong, Y. Tanaka, K. Murata, H. Aizaki, T. Wakita, Interleukin-1 and tumor necrosis factor-alpha trigger restriction of hepatitis B virus infection via a cytidine deaminase activation-induced cytidine deaminase (AID), J. Biol. Chem. 288 (2013) 31715–31727.
[15] S. Mita, H. Suzuki, H. Akita, H. Hayashi, R. Onuki, A.F. Hofmann, Y. Sugiyama, Inhibition of bile acid transport across Na+/taurocholate cotransporting polypeptide (SLC10A1) and bile salt export pump (ABCB 11)-coexpressing LLC-PK1 cells by cholestasis-inducing drugs, Drug Metab. Dispos. 34 (2006) 1575–1581.
[16] A. Schulze, K. Mills, T.S. Weiss, S. Urban, Hepatocyte polarization is essential for the productive entry of the hepatitis B virus, Hepatology 55 (2012) 373–383.
[17] M. Koyanagi, M. Hijikata, K. Watashi, O. Masui, K. Shimotohno, Centrosomal P4.1-associated protein is a new member of transcriptional coactivators for nuclear factor-kappaB, J. Biol. Chem. 280 (2005) 12430–12437.
[18] P. Gripon, I. Cannie, S. Urban, Efficient inhibition of hepatitis B virus infection by acylated peptides derived from the large viral surface protein, J. Virol. 79 (2005) 1613–1622.
[19] P. Gripon, C. Diot, A. Corlu, C. Guguen-Guillouzo, Regulation by dimethylsulfoxide, insulin, and corticosteroids of hepatitis B virus replication in a transfected human hepatoma cell line, J. Med. Virol. 28 (1989) 193–199.
[20] K. Watashi, A. Sluder, T. Daito, S. Matsunaga, A. Ryo, S. Nagamori, M. Iwamoto, S. Nakajima, S. Tsukuda, K. Borroto-Esoda, M. Sugiyama, Y. Tanaka, Y. Kanai, H. Kusuhara, M. Mizokami, T. Wakita, Cyclosporin A and its analogs inhibit hepatitis B virus entry into cultured hepatocytes through targeting a membrane transporter NTCP, Hepatology, in press.
[21] A. Schulze, P. Gripon, S. Urban, Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans, Hepatology 46 (2007) 1759–1768.
[22] O. Hantz, R. Parent, D. Durantel, P. Gripon, C. Guguen-Guillouzo, F. Zoulim, Persistence of the hepatitis B virus covalently closed circular DNA in HepaRG human hepatocyte-like cells, J. Gen. Virol. 90 (2009) 127–135.