Paclitaxel-induced Immune Dysfunction and Activation of Transcription Factor AP-1 Facilitate Hepatitis B Virus Replication

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Received: 29 November 2023 | Revised: 18 February 2024 | Accepted: 19 February 2024 | Published online: 06 March, 2024

Abstract

Background and Aims: Hepatitis B virus (HBV) reactivation is commonly observed in individuals with chronic HBV infection undergoing antineoplastic drug therapy. Paclitaxel (PTX) treatment has been identified as a potential trigger for HBV reactivation. This study aimed to uncover the mechanisms of PTX-induced HBV reactivation in vitro and in vivo, which may inform new strategies for HBV antiviral treatment. Methods: The impact of PTX on HBV replication was assessed through various methods including enzyme-linked immunosorbent assay, dual-luciferase reporter assay, quantitative real-time PCR, chromatin immunoprecipitation, and immunohistochemical staining. Transcriptome sequencing and 16S rRNA sequencing were employed to assess alterations in the transcriptome and microbial diversity in PTX-treated HBV transgenic mice. Results: PTX enhanced the levels of HBV 3.5-kb mRNA, HBV DNA, HBeAg, and HBsAg both in vitro and in vivo. PTX also promoted the activity of the HBV core promoter and transcription factor AP-1. Inhibition of AP-1 gene expression markedly suppressed PTX-induced HBV reactivation. Transcriptome sequencing revealed that PTX activated the immune-related signaling networks such as IL-17, NF-κB, and MAPK signaling pathways, with the pivotal common key molecule being AP-1. The 16S rRNA sequencing revealed that PTX induced dysbiosis of gut microbiota. Conclusions: PTX-induced HBV reactivation was likely a synergistic outcome of immune suppression and direct stimulation of HBV replication through the enhancement of HBV core promoter activity mediated by the transcription factor AP-1. These findings propose a novel molecular mechanism, underscoring the critical role of AP-1 in PTX-induced HBV reactivation.


Introduction

The continuing high prevalence of chronic hepatitis B virus (CHB) infection is a global public health concern, particularly in China and other countries in the Asia-Pacific region. Approximately 296 million people globally are believed to have CHB, and hepatitis B virus (HBV) infection-related illnesses led to approximately 820,000 fatalities in 2019.1,2 HBV infection can cause varying degrees of liver inflammation and fibrosis. In the absence of antiviral treatment, persistent CHB infection can lead to cirrhosis and hepatocellular carcinoma. HBV infection is amenable to early diagnosis and effective treatment, but the current diagnosis and treatment rates of HBV in China are only 22% and 15%, respectively.3 Despite the widespread implementation of hepatitis B vaccination and other interventions, the prevention and treatment of HBV still pose significant challenges. HBV is transported to the liver through bloodstream transmission and infects hepatocytes. After attaching to its functional receptor, sodium taurocholate cotransporting polypeptide...
tide (NTCP), the virus enters the host cell cytoplasm through endocytosis. Covalently closed circular DNA (cccDNA) is formed in the nucleoplasm by the release of relaxed circular DNA (rcDNA) from the nuclear capsid. HBV cccDNA is the primary source of sustained viral replication and transcription. It exists in a microchromosomal form for an extended period and is highly stable, acting as the persistent reservoir of the virus and a significant cause of HBV reactivation during immunosuppression. Even after HBV infection clearance and serologic conversion of HbsAg, the presence of HBV cccDNA can still be observed within the liver.

The definition of HBV reactivation may vary slightly among different guidelines, but the underlying concept remains the same. It refers to the activation of HBV in individuals with quiescent infection or those having low levels of viral replication. HBV reactivation is a frequent complication of immunosuppressive or antineoplastic therapy in individuals with chronic HBV infection leading to liver damage. The main underlying mechanism of HBV reactivation is the disruption of the balance between the virus and the host's immune function. Treatment with chemotherapeutic drugs or immunosuppressants can compromise the immune function of patients, leading to a significant increase in viral replication within the host. Subsequently, when these drugs are discontinued and the immune function is restored, an overly strong immune response can occur, resulting in hepatocyte injury. Of note, the likelihood of HBV reactivation is particularly high among individuals treated with B-cell depleting agents such as rituximab. However, in more than half of all cases, HBV reactivation occurs early in the course of chemotherapy, rather than during the interval after the completion of chemotherapy. This suggests that immune dysfunction alone cannot explain all the clinical phenomena, and it is possible that certain chemotherapeutic agents directly stimulate HBV replication.

Paclitaxel (PTX) is a widely utilized first-line therapeutic agent for various types of cancers. Its primary mechanism of action is to stabilize and enhance the polymerization of microtubule proteins, leading to the prevention of microtubule depolymerization and the inhibition of cell mitosis. However, clinical trials have reported cases of severe HBV reactivation leading to liver damage in breast cancer patients following PTX treatment.

While previous clinical studies have documented the reactivation of HBV in individuals concurrently diagnosed with HBV infection and tumors following PTX treatment, the direct impact of PTX on HBV itself remains a subject of controversy. Furthermore, there is a paucity of comprehensive investigations elucidating the molecular mechanisms underlying chemotherapeutic drug-induced HBV reactivation. Therefore, in this study, we conducted a series of in vitro and in vivo experiments to examine the relationship between PTX and HBV replication. The objective was to shed light on the potential mechanisms of PTX-induced HBV reactivation.

Methods

Cell culture

HepAD38 (HB-B065, ATCC, Manassas, VA, USA) cells were obtained from the American Type Culture Collection, while HepG2-NTCP cells were generously provided by Prof. Ning-shao Xia (Xiamen University, Fujian, China). HepG2.2.15 cells were stored in our laboratory. HepAD38 cells were cultured in a growth medium comprising Dulbecco's modified Eagle's medium (DMEM, HyClone, Logan, UT, USA) with the addition of 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 1 µg/mL tetracycline, 500 µg/mL G418, 100 units/mL penicillin (HyClone, Logan, UT, USA), and 100 µg/mL streptomycin (HyClone, Logan, UT, USA) as previously described. To boost NTCP receptor expression in HepG2-NTCP, an additional 2 µg/mL of doxycycline was added to the growth medium.

Antibodies and reagents

The following antibodies were used for immunoblot analysis: anti-hepatitis B core antigen (HBcAg) (B0586) from Dako (Glostrup, Denmark) and anti-GAPDH (Cat. no. 60004-1) from ProteinTech (Rosemont, IL, USA). Anti-Rabbit-HRP (ab6721) from Abcam (Cambridge, UK) and anti-c-Jun (Cat. no. 9165S) from Cell Signaling Technology (Danvers, MA, USA). Paclitaxel (PTX, Cat#HY-B0015) was obtained from MedChemExpress (Monmouth Junction, USA). Sangon Biotech (Shanghai, China) provided G418, tetracycline (A100422), and doxycycline (DOX).

Animal studies

HBV transgenic mice (HBV-Tg, C57BL/6), a gift from Xiamen University, were housed in the SPF of the Animal Centre of Chongqing Medical University. A total of 8 HBV-Tg mice, aged 6–8 weeks, were included in each group. Animal administration used in this study has been described in the previous study. The mice were administered intraperitoneal injections of phosphate-buffered saline (PBS, control) or PTX (10 mg/kg body weight) every other day for a total of seven injections. Half of the mice were sacrificed after the injection on the 14th day, and the remaining mice were kept under observation until one week after drug withdrawal. The liver tissue samples were harvested and quickly frozen in liquid nitrogen and stored at −80°C, together with the colon contents for sequencing purposes.

Western blot analysis

Total proteins were extracted from the cells using RIPA lysis buffer (Cat. no. C0005; Beyotime Biotechnology, Shanghai, China) with 1 mM phenylmethanesulfonyl fluoride (Beyotime Biotechnology, Shanghai, China). The protein samples were subjected to electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and then electrically transferred onto a PVDF membrane (IPVH00010, Merck Millipore, Billerica, MA, USA). The protein bands were detected using an enhanced chemiluminescence (ECL) detection kit (Beyotime Biotechnology, Shanghai, China).

RNA isolation and RT-qPCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. All operations were carried out under enzyme-free conditions. For RT-qPCR detection, cDNA synthesis was performed using modified Moloney murine leukemia virus reverse transcriptase (RR047A, TaKaRa, Tokyo, Japan). The SYBR Green qPCR Master Mix with special primers was utilized for RT-qPCR analysis on a Bio-Rad CFX Connect real-time PCR Detection System (Bio-Rad). The fold change was examined using the 2−ΔΔCt method with β-actin serving as the internal control (Supplementary File 1). All primers used in the study are listed in Supplementary Table 1.

Immunohistochemistry

Immunohistochemistry was performed as described. After fixation of liver tissues with 4% paraformaldehyde, paraffin-embedded sections were prepared according to the standard procedure and stored at room temperature. The tissue sections were incubated overnight with primary antibodies.
at 4 °C, followed by coating with biotin-labelled sheep anti-
mouse/-rabbit IgG polymer (ZSGB-BIO, Beijing, China) at
room temperature for 30 minutes. Diaminobenzidine solution
(ZLI-9019, ZSGB-BIO, Beijing, China) was applied for colour
development at the end. The Pannoramic Scan 250 Flash or
MIDI system was used to scan the stained slides, and the
Pannoramic Viewer 1.15.2 (3DHistech, Budapest, Hungary)
was employed for image acquisition.

**Dual-Luciferase reporter assay**

Briefly, dual-Luciferase reporter assay was performed as pre-
viously described. A luciferase reporter plasmid containing
the HBV core promoter Cp, X gene promoter Xp, and surface
antigen promoters Sp1 and Sp2 was constructed using pGL3-
Basic as a vector (constructed and preserved by the labo-
atory). Cells were co-transfected with plasmids pcDNA3.1,
TK-Rluc, and the corresponding HBV promoter plasmids
(pGGL3-Cp, pGGL3-Xp, pGGL3-Sp1, pGGL3-Sp2). After 48 hours
of incubation, the luciferase activity was quantified using the
Dual-Luciferase Reporter Gene Assay Kit (Promega, Madison,
WI, USA) according to the manufacturer’s guidelines.

**RNA sequencing**

Mouse liver tissues harvested from PTX and CON groups
were snap-frozen in liquid nitrogen and used for transcrip-
tome sequencing. RNA sequencing analysis was conducted at
Shanghai Majorbio Bio-Pharm Technology Co. Ltd. (Shang-
hai, China) following the manufacturer’s descriptions. Total
RNA was extracted using TRizol® Reagent and the mRNA
was purified using oligo (dT) beads for library construction.
The paired-end RNA sequencing library was subjected to se-
quencing using the Illumina NovaSeq 6000 sequencer, with
a read length of 2×150 bp, to generate the sequencing data.

**16S rRNA sequencing**

Samples of intestinal contents were collected and total DNA
was extracted. PCR was employed to amplify the 16S rRNA of
cancer present in the samples. The resulting PCR products
were detected by agarose gel electrophoresis. Based on the
initial quantitative analysis of the electrophoresis results, the
PCR products were measured using the fluorescence-based
quantification method. Subsequently, high-throughput se-
quencing was conducted using the Illumina platform. The
sequence of 338F was 5′-ACTC CTACGGGAGGCAGCAG-3′, and
the sequence of 806R was 5′-GGACTACHVGGGTWTCT AAT-
3′. The primer sequences have been previously reported.

**Statistical analysis**

GraphPad Prism 8.0 was used for statistical analyses. For con-
tinuous variables, mean (±standard deviation) values from three independent experiments are presented. Inter-
group comparisons were conducted using the t-test, while one-
way ANOVA was used for multi-group comparisons. P
values <0.05 were considered indicative of statistical signifi-
cance.

**Results**

**Paclitaxel directly stimulates HBV replication in vitro**

We utilized the stable HBV-expression hepatocellular carci-
noma (HCC) cell line (HepAD38, HepG2.2.15) and the HBV
naturally-infected cell model (HepG2-NTCP) to investigate
whether PTX has a direct effect on HBV replication in vitro.
Initially, we assessed the cytotoxicity of PTX within a specific
concentration range (0–4 µM) and found that it had a low
cytotoxic effect, with cell viability >80% (Supplementary Fig.
1A). Furthermore, the IC50 value of PTX was determined us-
ing the CCK8 assay kit. The IC50 value of PTX in HepAD38,
HepG2-NTCP, and HepG2.2.15 was 4.648 µM, 5.562 µM, and
6.489 µM, respectively (Supplementary Fig. 1B). To assess
the impact of PTX on HBV replication within a secure range,
we incubated HepAD38 and HepG2-NTCP cells with PTX at
concentrations ranging from 0 to 4 µM.

Following treatment of cells with PTX at various concen-
trations (0–4 µM) for 120 hours, the intracellular HBV 3.5-
kb mRNA levels (Fig. 1A), HBV DNA levels (Fig. 1B), and
HBV cccDNA levels (Fig. 1C) were significantly increased in
a concentration-dependent manner. The intracellular HBcAg
expression was similarly increased (Fig. 1D–E). Enzyme-
linked immunosorbent assay (ELISA) of cell culture super-
natants showed that PTX treatment enhanced HBcAg and
HBsAg secretion levels (Fig. 1F and Supplementary Fig. 2A)
in a concentration-dependent manner. Similar findings were
observed in HepG2.2.15 cells (Supplementary Fig. 2B–F).
These findings indicated that PTX may potentially enhance
HBV replication in vitro.

**Paclitaxel can promote HBV replication in vivo**

The impact of PTX on HBV replication in vivo was investi-
gated using HBV-Tg mice. HBV-Tg mice were divided into 2
groups (n=8). Intraperitoneal injection of PTX (10 mg/kg)
was administered in the PTX group while PBS was admin-
istered in the control group (Con). The injections were ad-
ministered every other day for a total of seven injections
(Fig. 2A). The observation time was extended to one week
after the withdrawal of the drug. Serum samples of the mice
were collected on the 7th, 14th, and 21st day, respectively.
PTX administration resulted in a significant increase in serum
HBV DNA levels, along with increased levels of HBeAg and
HBsAg (Figure. 2B–D), but their secretion levels showed a
slight decrease one week after drug withdrawal. HBV 3.5-kb
mRNA, HBV DNA, and HBV cccDNA levels in the liver were
also significantly increased (Fig. 2E–G). Immunohistochemi-
cal staining showed a significant increase in HBCAg and HB-
sAg protein expression in hepatocytes (Fig. 2H), which
was correlated with the duration of drug action, and the replica-
tion levels were slightly decreased after drug withdrawal. In
conclusion, PTX treatment significantly enhanced HBV repli-
cation levels in HBV-Tg mice. We also detected serum levels
of alanine aminotransferase (ALT) and aspartate aminotrans-
ferase (AST) levels to explore whether PTX-mediated HBV
reactivation would induce liver injury. Our findings suggested
no significant damaging effect of PTX on the liver of HBV
transgenic mice (Supplementary Fig. 2G–H). We speculated
that the time point of our observations may be in the active
phase of HBV replication, which has not yet progressed to the
phase of hepatic impairment.

**Paclitaxel treatment induces altered gene expression in
HBV-Tg mice**

We performed liver transcriptome analysis to further ex-
plorthe molecular mechanism of PTX-induced HBV reac-
tivation. The PCA plot (Fig. 3A) showed clustering of sam-
pies in the Con and PTX groups with a clear separation
of samples in each group. This indicated the repeatability
of samples in each group (Fig. 3B). KEGG enrichment analysis of differentially
expressed genes (DEGs) revealed notable enrichment of
various biological processes, including cancer:overview,
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PTX enhances HBV replication in vitro. (A–C) qPCR assay was employed to quantify the levels of HBV 3.5-Kb mRNA, HBV DNA, and HBV cccDNA in both HepAD38 cells and HepG2-NTCP cells following PTX treatment. (D–E) Immunoblot analysis of HbcAg expression levels in HepAD38 cells and HepG2-NTCP cells. Den- sitometry analysis was used to measure the relative levels of HbcAg. (F) Results of ELISA showing HbeAg levels in the culture medium supernatants of HepAD38 cells and HepG2-NTCP cells. Den-}

infectious disease: viral, immune system and signal trans-duction (Fig. 3C–D). Further analysis revealed that PTX upregulates immune response-related pathways such as the IL-17 signaling pathway, NF-Kappa B signaling path-way, and MAPK signaling pathway (Fig. 3E). The heatmaps depicting the enrichment of the DEGs in the three immune response pathways are presented in Figure 3F–H. Transcription factor family statistics were also performed on the DEGs. The results showed that they could be clustered in the Fos related Jun related transcription factor family (Fig. 3I), including Jun, Fosl2, and Jund. Jun is a member of the AP-1 family, and AP-1 has been reported to have a significant impact on HBV replication.17

**AP-1 activation can stimulate HBV replication**

HBV replication is known to be finely regulated by a wide range of liver-enriched transcription factors. Therefore, we screened the transcription factors involved in the regulation of HBV expression and analyzed their expression levels after PTX treatment. We observed a remarkable increase in the mRNA levels of transcription factors AP-1 and CREB1 (Fig. 4A, Fig. 3A). Notably, AP-1 exhibited a greater increase, which was consistent with the results of transcriptome se-}

the c-Jun subunit of AP-1 was also significantly increased (Fig. 4B, Supplementary Fig. 3B). Previous in vitro investi-gations have shown that PTX induces upregulation of AP-1 mRNA and protein levels, and we verified whether PTX pro-motes the enhancement of AP-1 activity in HBV-Tg mice. Immunohistochemistry showed a significant increase in AP-1 protein levels in the liver tissues of mice on the 14th day of PTX administration and one week after withdrawal of the drug (day 21) as compared to the PBS control (Fig. 4C). Based on these findings, we selected AP-1 as the target for further investigation in subsequent experiments. To validate the role of AP-1 in HBV replication, we employed siRNA tar-geting to silence AP-1 expression (Fig. 4D–E, Supplemen-}

**PTX enhances viral HBV replication by activating the binding of AP-1 to HBV core promoter**

Numerous transcription factors can bind to HBV promoter/
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Enhancer elements to regulate HBV transcription and replication. We found that PTX induced AP-1 activation to promote HBV transcription and replication, but the specific mechanism by which AP-1 regulates HBV transcription and replication is not clear. We initially assessed the impact of PTX on the activity of four HBV promoters: HBV Cp, HBV Xp, HBV Sp1, and HBV Sp2. The findings indicated a notable enhancement in the activity of the core promoter (Fig. 5A, Supplementary Fig. 3E). Silencing of AP-1 affected the activity of the HBV core promoter (Fig. 5B, Supplementary Fig. 3F), while CHIP assays suggested an interaction between AP-1 and the HBV core promoter (Fig. 5C, Supplementary Fig. 3G). Because si-

Fig. 2. PTX administration promotes viral biosynthesis in vivo. (A) Schematic illustration of the PTX treatment regimen in HBV transgenic mice. (B) HBV DNA levels in mouse serum were quantified using qPCR. (C–D) HBsAg and HBcAg levels in mouse serum were measured using ELISA. (E–G) The levels of HBV 3.5-Kb mRNA, HBV DNA, and HBV cccDNA in liver tissues were determined using qPCR. (H) Immunohistochemistry analysis of HBcAg and HBsAg expression in liver tissues (scale bar: 60 µm). Mean±SD values from three independent experiments are presented. *p<0.05, **p<0.01, and ***p<0.001 vs. PBS control. HBV, hepatitis B virus; HBV-Tg mice, HBV-transgenic mice; PTX, paclitaxel; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV cccDNA, HBV covalently closed circular DNA.
Fig. 3. Differentially expressed genes (DEGs) in the liver tissues of PTX-treated (PTX) mice and control (Con) mice. (A) Principal component analysis (PCA) of transcriptional analysis of liver tissue of mice in Con and PTX groups. (B) Statistical chart of significantly up- and down-regulated genes (P-value < 0.05 and at least twofold change) in Con vs PTX. (C–D) The distribution of Kyoto Encyclopedia of Genes and Genomes (KEGG) terms for different pathways assigned to significantly up-regulated and down-regulated genes. (E) The top 20 significantly changed pathways analyzed by KEGG pathway enrichment. (F–H) Heatmap of three immune-related pathways enriched in the DEGs. (I) Statistical chart of transcription factor family among the Con and PTX groups. PCA, Principal component analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes.
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PTX-induced activation of AP-1 facilitate HBV replication, we further explored whether PTX promotes HBV replication by modulating AP-1 activity and thereby promoting HBV replication. The promotion of HBV 3.5-kb mRNA, HBV DNA, and HBV cccDNA expression levels following AP-1 silencing. (1) Quantitative analysis of ELISA for HBeAg and HBsAg levels in HepAD38 cells following AP-1 silencing. **p < 0.001. PTX, paclitaxel; AP-1, activator protein 1; HBV, hepatitis B virus; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV cccDNA, HBV covalently closed circular DNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Dysbiosis of gut microbiota induced by PTX treatment

To assess the effects of PTX administration on the gut microbiota, we performed 16S rRNA sequencing. The results of the rarefaction curve of the Sobs index at the ASV level showed a leveling-off of the rarefaction curve for both the Con and PTX groups, with sufficient sequencing volume to cover the microbial communities in all samples (Fig. 6A). The analysis of α-diversity, as measured by the Chao1 index and Shannon index, showed no significant differences between the groups (Fig. 6B–C). The results of the hierarchical clustering tree
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Fig. 5. PTX enhances viral HBV replication by activating the binding of AP-1 to HBV core promoter. (A) HepG2-NTCP cells treated with 4 µM PTX for 48 h after 12 h of transfection with HBV core, PreS1, PreS2, or X promoter luciferase reporter vectors. (B) Dual-luciferase reporter assays for detecting the effect of silencing AP-1 on HBV promoter activity. (C) CHIP-qPCR assay was used to detect the interaction between AP-1 and HBV core promoter. (D–F) Quantitative analysis of qPCR results showing the effect of silencing AP-1 on the PTX regulation of 3.5-kb mRNA, HBV DNA, and HBV cccDNA levels in HepAD38 cells. (G–H) ELISA analysis of the effect of silencing AP-1 in HepAD38 cells on PTX regulation of HBeAg and HBsAg levels. (I) Immunoblot analysis of HBcAg expression levels in AP-1 silenced HepAD38 cells. +, siAP-1 or PTX; –, siControl or DMSO. (J–L) HepG2 cells were transfected with pGEM-HBV1.3 or pGEM-HBV1.3MUT, and then treated with 4 µM PTX. qPCR assay was employed to quantify the levels of HBV 3.5-Kb mRNA, HBV DNA, and HBV cccDNA. (M–O) ELISA assays were used to detect HBeAg and HBsAg levels in the culture medium supernatant. (O) Immunoblot analysis of HBcAg expression levels in HepG2 cells. Mean±SD values from three independent experiments are presented. *p<0.05, **p<0.01, ***p<0.001.

PTX, paclitaxel; HBV Cp, HBV core promoter; HBV Xp, HBV X promoter; HBV Sp1, HBV pre S1 promoter; HBV Sp2, HBV pre S2 promoter; AP-1, activator protein 1; HBV, hepatitis B virus; WT, wild type; Mut, mutant; HBcAg, hepatitis B core antigen; HbeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
at the ASV level showed significant clustering of the colony structure in both Con and PTX groups (Fig. 6D). The PCoA plot (Fig. 6E), also built from the heterogeneity matrix using the Bray-Curtis index to explain the β-diversity, showed individual clustering between the groups, showing significant differences at the ASV level. These results indicated that PTX treatment altered the composition of the gut microbiota in HBV-Tg mice. A total of 354 ASVs were shared in both groups, while 213 and 239 ASVs were exclusively found in Con and PTX groups, respectively (Fig. 6F). The relative abundance of bacteria was assessed at both the phylum and genus levels (Fig. 6G–H). Using the Kruskal-Wallis rank-sum test, we observed a significant increase in the relative abundance of *Campylobacterota* and *Deferribacterota* at the phylum level in the PTX group compared to the Con group. Conversely, the relative abundance of *Actinobacterota* was significantly lower in the PTX group (Fig. 6I). At the genus level, the proportional representation of detrimental bacteria such as *Escherichia-Shigella, Helicobacter,* and *unclassified_o__Oscillospirales* was remarkably elevated in the PTX group, while the proportional representation of advantageous bacteria such as *norank_f__Muribaculaceae, Dubosiella, Allobaculum,* and *norank_f__Lachnospiraceae, Enterohabdus* were considerably reduced.

Fig. 6. Changes in the gut microbiota of HBV-Tg mice following treatment with PTX. (A) Rarefaction curve at the ASV level; (B–C) Chao index and Shannon index calculated at the ASV level; (D–E) The β-diversity of ASV-level microbial community was evaluated using hierarchical clustering (D) and principal coordinate analysis (PCoA) I. (F) Venn diagram of common and peculiar ASVs in the two groups. (G–H) The relative occurrence of bacteria at the phylum (G) and genus (H) levels. (I–J) Comparative analysis at the phylum level (I) and genus level (J). *p < 0.05, **p < 0.01. PCoA, principal coordinate analysis; Con, control; PTX, paclitaxel.
Discussion

The persistence of HBV cccDNA within the nucleus of infected hepatocytes provides the virological foundation for HBV reactivation. Disruptions in the balance between viral replication and host immune control, resulting from alterations in either the virus or the immune system, can lead to HBV reactivation. Immunosuppression induced by antineoplastic drugs may impair control of HBV replication. It may also directly promote HBV replication, leading to HBV reactivation.\(^1\) Immunomodulators, such as corticosteroids, not only inhibit the function of cytotoxic T-cells but also activate regulatory elements within the HBV gene in cultured human HCC cells, thereby promoting HBV replication.\(^1\) Cisplatin-induced autophagy was found to enhance HBV replication by triggering the ROS/JNK pathway and inhibiting the Akt/mTOR signaling pathway.\(^2\) The present study demonstrates that PTX may activate HBV core promoter activity, thereby enhancing HBV replication through the activation of the transcription factor AP-1. Furthermore, we conducted transcriptome sequencing and 16S rRNA sequencing to perform a comprehensive quantitative analysis of the changes in the transcriptome and microbial diversity of HBV-Tg mice following PTX treatment, which may reduce host resistance to HBV infection.

PTX is a class of cytotoxic drugs commonly used in the management of diverse cancer types, including advanced ovarian cancer, metastatic breast cancer, and non-small cell lung cancer.\(^2\) It is often used in combination with other chemotherapeutic agents or hormones. PTX has been reported as one of the chemotherapeutic agents associated with HBV reactivation.\(^3\) Trastuzumab in combination with PTX was found to increase the risk of HBV reactivation.\(^4\) A case report documented PTX-induced HBV reactivation in a breast cancer patient who eventually died due to severe liver and kidney failure.\(^5\) However, there is no clear consensus on whether HBV reactivation occurs as a result of the underlying disease itself, the PTX treatment, or the interaction between the two factors.\(^6\) In the present study, PTX was found to directly promote HBV replication in vitro in a concentration-dependent manner. The in vivo study using HBV-Tg mice also suggested that PTX promotes HBV reactivation.

In the present study, PTX treatment increased the activity of the HBV core promoter. Further investigation identified AP-1 as a potential regulator of the HBV core promoter in response to PTX. The AP-1 transcription factor is composed of Jun proteins (c-Jun, JunB, and JunD), Fos proteins (c-Fos, FosB, Fra-1, and Fra-2), as well as ATF and MAF proteins, forming a dimeric complex.\(^6\) Activation of AP-1 has been linked to different aspects of cancer biology, such as apoptosis, metastasis, cell growth, angiogenesis, invasion, and drug resistance.\(^7\) The transcription factor AP-1 plays a crucial role in many aspects of liver biology. For instance, treatment with nitidine chloride was shown to induce mitochondrial damage and apoptosis in hepatocytes through the activation of the JNK/c-Jun signaling pathway.\(^7\) Additionally, the histone methyltransferase SETD2 was found to induce the activation of c-Jun/AP-1 in the liver by promoting the accumulation of lipids, thereby contributing to hepatocellular carcinogenesis.\(^7\) AP-1 has also been implicated in the pathogenesis of viral hepatitis,\(^7\) as HBX interacts with Jab1 to enhance AP-1 activation.\(^7\) AP-1 has been shown to bind to the Enh II/Xp region of the HBV genome.\(^7\) Additionally, AP-1 binding motifs have been identified in the Enh II/Cp region of HBV.\(^7\) In the present study, PTX-mediated activation of AP-1 had no significant effect on HBV X promoter activity. PTX-mediated HBV reactivation is a complex process, and the activity of the HBV X promoter may also be regulated by multiple factors, possibly by transcription factors other than AP-1. Our results offer additional support for the role of AP-1 in controlling HBV replication and its involvement in modulating the activity of the HBV core promoter upon PTX treatment.

For an in-depth characterization of the mechanism underlying the regulation of HBV by PTX, we performed additional investigations by integrating transcriptome and gut microbiota sequencing. Transcriptome sequencing results revealed significant changes in biological processes such as viral infectious diseases, immune system, and signal transduction after PTX treatment. We detected enrichment of immune response-related DEGs in the liver, such as the IL-17 signaling pathway, NF-KappaB signaling pathway, and MAPK signaling pathway,\(^8\) which are critical modulators of the immune response. Previous studies have indicated that immunosuppressive agents or chemotherapy drugs can induce immune system dysregulation, leading to HBV reactivation. Transcriptome sequencing suggested that PTX not only directly promotes HBV replication but also enhances HBV replication by inducing immune dysfunction and inflammatory responses. PTX-induced HBV reactivation may be a combined result of immunosuppression and direct enhancement of HBV replication. Additionally, our analysis of transcription factor families in liver DEGs revealed a concentration of Fos related::Jun related transcription factor families, which were consistent with our in vitro results. The gut microbiota has a pivotal influence on the maintenance of intestinal homeostasis and liver health via the intricate interplay of the gut–liver axis,\(^9\) and dysbiosis of the gut microbiota is associated with immune imbalance.\(^9\) Emerging research has revealed a correlation between dysbiosis of gut microbiota and the onset and progression of viral hepatitis.\(^9\) We observed significant differences between the PTX and Con groups with respect to the structural model and composition within the colon. The relative occurrence of beneficial bacteria was significantly increased in the PTX group, while the relative occurrence of beneficial bacteria was significantly decreased. This suggested that PTX-induced dysbiosis of the gut microbiota may be one of the reasons for immune imbalance, ultimately leading to enhanced HBV replication.

Conclusion

In this study, PTX treatment was found to directly promote HBV replication and transcription, leading to HBV reactivation in HBV stable expression cell models, HBV natural infection cell models, and HBV transgenic mouse models. Further molecular analysis indicated that PTX may enhance HBV replication and transcription through the promotion of HBV core promoter activity mediated by the transcription factor AP-1. Furthermore, PTX treatment was found to induce immune system dysregulation and dysbiosis of the gut microbiota, which may potentially contribute to the induction of HBV reactivation. However, it is important to acknowledge that our experimental models may not fully replicate the complex dynamics of HBV infection and reactivation observed in clinical patients. Therefore, more comprehensive and in-depth research is required to provide references for the development of prevention and treatment strategies for clinical HBV reactivation.

Funding

This research received financial support from various sources including the Innovation and Development Joint Fund of Chongqing Natural Science Foundation (Grant number CSTB2023NSQC-LZX0099), Chongqing Science and Health Joint Medical High-end Talent Project (Grant No. 2022GDRC012),
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Chongqing Biomedical R&D Major Special Project (Grant No. CSTB2022TIAAD-STX0013), Science and Technology Research Program of Chongqing Municipal Education Commission (Grant No. KJZD-K202100402), CQMU Program for Youth Innovation in Future Medicine (Grant No. W0073), and CQMU Program for Research Program of Chongqing Municipal Education Commission (Grant No. KJZD-K202100402), CQMU Program for Innovation (Grant No. W0073), and SW. JG, LC, and XL provided materials and technical support. SC, WL, and QY critically revised the manuscript. MH, and SW. JG, LC, and XL provided materials and technical support. SC, WL, and QY critically revised the manuscript. The manuscript was drafted by SC, LC, and XL. AR was responsible for developing the methodology. The study was supported by Inducing Cell Cycle Arrest. J Clin Transl Hepatol 2021;19(2):160–165. doi:10.14218/JCTH.2021.00105, PMID:34007797.


Joint Project(Grant No. 2020XYLH-021). Youn Innovation in Future Medicine (Grant No. W0073), and SW. JG, LC, and XL provided materials and technical support. SC, WL, and QY critically revised the manuscript. The manuscript was drafted by SC, LC, and XL. AR was responsible for developing the methodology. The study was supported by Inducing Cell Cycle Arrest. J Clin Transl Hepatol 2021;19(2):160–165. doi:10.14218/JCTH.2021.00105, PMID:34007797.


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