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AZD8055 inhibits the secretion of HBV-related antigens through an autophagy-related mechanism

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ABSTRACT: Hepatitis B virus (HBV) affects approximately 300 million people worldwide, leading to chronic liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). Despite available treatments like interferon-alpha and nucleoside analogs, these do not eliminate HBV covalently closed circular DNA (cccDNA) from host cells. This study investigates the efficacy of AZD8055, a dual inhibitor of mTORC1 and mTORC2, in reducing HBV antigen secretion in HepG2 and Huh-7 HCC cells. Our findings indicate that AZD8055 significantly decreases both intracellular and extracellular levels of Hepatitis B surface antigen (HBsAg) and Hepatitis B e antigen (HBeAg) without compromising cell viability. This inhibition is mediated through the action of AZD8055 on the mTOR pathway, which enhances autophagic activity, a process reversible by autophagy inhibitors: 3-methyladenine (3-MA) and chloroquine (CQ). The results suggest AZD8055 as a potential novel therapeutic approach for HBV, potentially in combination with existing treatments, meriting further clinical investigation to validate these promising preclinical outcomes.

KEYWORDS: AZD8055, HBV, mTOR, autophagy

INTRODUCTION

HBV is a major cause of chronic hepatitis, affecting around 300 million people globally [1] . Even with the presence of effective vaccines, HBV continues to be widespread, leading to chronic infections that can cause liver inflammation, cirrhosis, and HCC, due to the integration of the viral genome into the host DNA. HBV is a small, enveloped DNA virus that infects liver cells by attaching to the sodium taurocholate cotransporting polypeptide (NTCP) receptor [2] . Once inside the cell, the viral nucleocapsid releases its relaxed circular DNA (rcDNA) into the cytoplasm, which is then transported to the nucleus and converted into the cccDNA by host DNA repair enzymes [3]. This cccDNA acts as a template for all HBV RNA transcripts, including pregenomic RNA (pgRNA), which is crucial for viral replication and protein production.

Currently, there are 2 main types of anti-HBV agents available: interferon (IFN-α) and nucleoside analogs (NAs). IFN-α interferes with HBV replication at various stages of its life cycle through antiviral and immunomodulatory effects but has limited effectiveness and significant side effects [4]. NAs work by inhibiting reverse transcription, reducing the synthesis of HBV DNA from pgRNA, and thereby suppressing new virus production. However, NAs cannot eliminate the cccDNA within infected hepatocytes or prevent the production of HBV proteins from integrated viral genomes [5]. Therefore, novel therapeutic strategies targeting HBV replication are urgently needed.

The mammalian target of rapamycin (mTOR) pathway is a crucial regulator of cellular growth, proliferation, and survival, influenced by various environmental factors such as nutrient availability, growth factors, and cellular energy levels [6]. This pathway operates through 2 distinct complexes, mTORC1 and mTORC2, each with specific roles in cellular processes. mTORC1 predominantly regulates protein synthesis and autophagy, while mTORC2 is involved in cell survival and the organization of the cytoskeleton [7]. Significant evidence suggests a complex interaction between the mTOR pathway and HBV replication [8]. Studies have indicated that mTOR inhibitors such as rapamycin can both promote and impede HBV replication. They may inhibit HBV replication by reducing the activity of transcription factors like PGC1α and PPAR or by enhancing autophagy [9–11]. Conversely, rapamycin is also reported to suppress HBV replication by promoting the degradation of the HBx protein [12]. This contradictory role of mTOR inhibitors in influencing HBV replication highlights the need for further investigation to elucidate these mechanisms comprehensively.

AZD8055 is a potent and selective inhibitor of the mTOR that has gained attention for its therapeutic potential in cancer treatment [13]. Unlike earlier mTOR inhibitors that generally target only the

mTORC1 complex, AZD8055 is designed to inhibit both mTORC1 and mTORC2 complexes, offering a broader inhibition of the mTOR signaling pathway. This dual inhibition is significant because mTORC2 has been implicated in the activation of AKT, promoting survival and resistance mechanisms in cancer cells [14]. However, the impact of AZD8055 on HBV replication has not been previously reported. In this study, we discovered that AZD8055 significantly inhibits the secretion of HBsAg and HBeAg without affecting cell viability. Furthermore, we observed that AZD8055 also reduces the intracellular expression of HBsAg and HBeAg. Notably, AZD8055 does not affect HBV transcription; instead, it influences HBsAg expression through post-transcriptional regulation. Further investigation revealed that AZD8055 significantly suppresses the activation of the mTOR pathway and enhances autophagic flux. When autophagy was blocked using autophagy inhibitors, the suppression of HBV-related antigen expression by AZD8055 was abolished. These findings suggest that AZD8055 promotes the autophagic degradation of HBsAg by inhibiting the mTOR pathway, highlighting its potential as a novel therapeutic strategy against HBV infection.

MATERIALS AND METHODS

Cell culture and transfection

All cell cultures were maintained at 37 °C within a humidified atmosphere containing 5% CO_2. Huh-7 and HepG2 cells were cultured in DMEM (Sangon Biotech, Shanghai, China) supplemented with 10% inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco, New York, USA). Transient transfection of plasmid pHBV1.2 was performed using the Lipofectamine 3000 Transfection Kit (Invitrogen, California, USA) following the manufacturer's instructions. After 6 h, the medium was aspirated, and cells were washed with PBS and replaced with fresh medium or medium containing various concentrations of AZD8055 for an additional 48 h.

Drugs and primary reagents

Small molecule compounds, AZD8055 (HY-10422), chloroquine (CQ, HY-17589A), and the PI3KC3 inhibitor 3-methyladenine (3-MA, HY-19312), were sourced from MedChemExpress, New Jersey, USA. The 10 mM stock solutions of AZD8055 and CQ were prepared in DMSO (Solarbio, Beijing, China) and stored at −20 °C. A 25 mM stock solution of 3-MA was prepared in DMEM complete medium and stored at −80 °C. Diagnostic kits for HBsAg and HBeAg (enzyme-linked immunosorbent assay, ELISA) were acquired from Beijing Wantai Biological Pharmacy, Beijing, China. Primary antibodies used were Anti-DDDDK-tag mAb (MBL, FLA-1, Beijing, China), P-4EBP1 (Abclonal, AP0030, Wuhan, China), 4EBP1 (Servicebio, GB115606, Wuhan, China), LC3 (Abclonal, A19665), β-actin (Abclonal, AC006), S6K1 (Cell Signaling, 9205S, Shanghai, China), P-S6K1 (Cell Signaling, 9234T), and P62 (Proteintech, 18420-1-AP, Wuhan, China). Secondary antibodies included Goat anti-Rabbit IgG (H+L) HRP (Invitrogen, 31460) and Goat anti-Mouse IgG (H+L) HRP (Invitrogen, 31430).

Table 1 Primer sequences for quantitative real-time PCR.

Name	Sequence
hβ-actin-F	CATGTACGTTGCTATCCAGGC
hβ-actin-R	CTCCTTAATGTCACGCACGAT
pgRNA-F	GCCTTAGAGTCTCCTGAGCA
pgRNA-R	GAGGGAGTTCTTCTTCTAGG
HBV Total RNA-F	ACGTCCTTTGTTTACGTCCCGT
HBV Total RNA-R	CCCAGCTCCTCCCAGTCCTTAA

h: human.

Cell proliferation analysis

Cell proliferation was assessed using the Cell™ Counting Kit-8 (CCK-8, EnoGene, E1CK- 000208-5, Nanjing, China) in accordance with the manufacturer's guidelines. The 5 mg/ml working solution of the CCK-8 reagent was prepared in serum-free DMEM. The cell supernatant was then collected, washed with PBS, and incubated with the CCK-8 solution at 37 °C in the dark for 40 min. Absorbance was measured at 450 nm.

Western blot analysis

Proteins from the whole-cell lysates prepared using RIPA buffer were separated by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto PVDF membranes. The membranes were blocked with 5% skim milk for 1 h, incubated overnight at 4 °C with primary antibodies on a shaker, and washed 3 times with TBST, prior to incubation with (1:4000) HRP-conjugated secondary antibodies for 1 h, followed by additional washes. The immunoreactive bands were visualized using appropriate chemiluminescent substrates. Densitometry analysis was performed using ImageJ software for protein quantification with β-actin being a loading control to ensure accurate measurement of protein expression levels across different samples.

Enzyme-linked immunosorbent assay (ELISA)

The levels of HBsAg and HBeAg in both cell culture supernatants and cell lysates were quantified using the Hepatitis B Virus Surface Antigen and e Antigen Diagnostic Kits (ELISA), following the procedures outlined in the accompanying manual.

RNA extraction and real-time fluorescence quantitative PCR

Intracellular HBV RNA was extracted using the EZ-press RNA Purification Kit (EZBioscience, B0004D, Roseville, USA) and reverse transcribed into cDNA

using ABScript Neo RT Master Mix for qPCR with gDNA Remover (ABclonal, RK20433). HBV RNA levels were quantified by real-time fluorescence quantitative PCR (RT-qPCR) using specific primers for pgRNA and HBV total RNA. Primers for RT-qPCR were listed in Table 1. β-actin mRNA levels were used as internal control. The fold change was analyzed using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.1 software. Results were expressed as mean ± standard deviation (mean ± SD). An unpaired *t*-test was used for comparisons between 2 groups, and one-way ANOVA was utilized for multiple group comparisons. A p-value of < 0.05 was considered statistically significant.

RESULTS

AZD8055 inhibits the secretion of HBsAg and HBeAg in hepatoma cells

To evaluate the impact of AZD8055 on HBV replication, a transient transfection model of HCC cells with the pHBV1.2 plasmid was utilized. In HepG2 cells, it was observed that AZD8055 at a concentration of 1 μM significantly reduced the levels of HBsAg and HBeAg in the cell supernatant. CCK-8 analysis indicated that concentrations of AZD8055 up to 1 μM did not affect cell viability (Fig. 1a). In another HCC cell line, Huh-7 cells, AZD8055 at just 0.1 μM was found to significantly inhibit the levels of HBsAg and HBeAg in the supernatant. CCK-8 results showed that 0.1 μM AZD8055 did not significantly affect the viability of Huh-7 cells, but 1 μM AZD8055 significantly reduced cell viability (Fig. 1b). These results suggest that AZD8055 significantly inhibits the secretion of HBsAg and HBeAg without affecting cell viability.

AZD8055 also inhibits the intracellular expression of HBsAg and HBeAg

To clarify the mechanism by which AZD8055 inhibits the secretion of HBsAg, we first investigated whether AZD8055 blocks the secretion process of HBsAg and HBeAg. By assessing the levels of HBsAg and HBeAg both intracellularly and in the cell supernatant, we discovered that AZD8055 inhibits the expression of HBsAg and HBeAg both inside the cells and in the supernatant. Moreover, the efficiency of AZD8055 in inhibiting both intracellular and supernatant HBsAg and HBeAg was similar (Fig. 2a,b). These results indicate that AZD8055 inhibits the secretion of HBsAg without affecting the HBsAg secretion pathway.

AZD8055 inhibits HBsAg expression via post-transcriptional regulation

Next, we assessed whether AZD8055 affects the mRNA levels of HBV. Through RT-qPCR analysis, we found that AZD8055 neither affects the expression of pgRNA nor the total RNA of HBV (Fig. 3a,b). This indicates that AZD8055 does not inhibit the expression of HBsAg at the mRNA level of HBV. Subsequently, we used a CMV promoter-driven, Flag-tagged HBsAg overexpression plasmid. This plasmid, utilizing a ubiquitous promoter, eliminates the interference from HBV transcriptional processes, thereby allowing us to explore whether AZD8055 inhibits HBsAg expression through post-transcriptional regulation. Western blot results demonstrated that AZD8055 significantly suppresses the expression of Flag-HBsAg (Fig. 3b,c), suggesting that AZD8055 can inhibit HBsAg expression via post-transcriptional mechanisms.

AZD8055 inhibits the mTOR pathway and activates autophagy flux

As previously mentioned, AZD8055 is an mTOR inhibitor. We investigated whether AZD8055 inhibits the activation of downstream signaling of the mTOR pathway in the context of HBV. As shown in Fig. 4a–c, AZD8055 significantly inhibited the phosphorylation of S6K1 and 4-EBP1, indicating a significant suppression of downstream mTOR signaling in the presence of HBV. The autophagy pathway significantly regulates HBV replication; studies have shown that early autophagy can promote HBV replication, while late-stage autophagy leads to the degradation of HBV-related proteins, thereby inhibiting HBV replication. Activation of the mTOR pathway inhibits the autophagy pathway, whereas inhibition of mTOR activates autophagy. Numerous studies have demonstrated that AZD8055 can activate cellular autophagy. Our experimental results also indicate that AZD8055 significantly increases the ratio of the autophagy activation marker LC3II/LC3I and markedly decreases the levels of the autophagy degradation marker P62 (Fig. 4d–f). These findings suggest that AZD8055 can inhibit the mTOR pathway and activate autophagic flux in the context of HBV.

Autophagy inhibitors, 3-MA and CQ, block the inhibitory effect of AZD8055 on HBsAg expression

Subsequently, we treated cells with the early autophagy inhibitor, 3-MA, and the late-stage autophagy inhibitor, CQ. We found that both 3-MA and CQ could block the ability of AZD8055 to inhibit the secretion of HBsAg and HBeAg (Fig. 5a,b). These results indicate that the suppression of HBsAg and HBeAg secretion by AZD8055 is dependent on the autophagy pathway.

DISCUSSION

The persistence of HBsAg is a pivotal factor in the establishment and maintenance of chronic HBV infection, positioning it as a prime target for therapeutic strategies aimed at eradicating HBV [15]. Various approaches, including immunotherapeutic strategies that amplify HBsAg-specific immune responses and agents

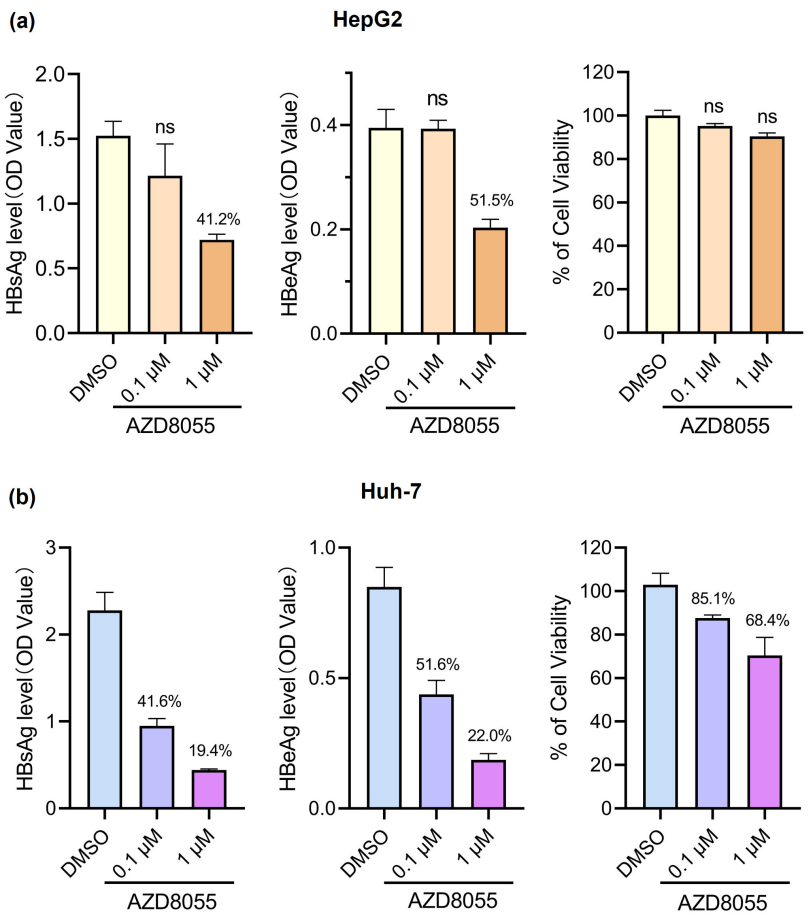

Fig. 1 AZD8055 inhibiting the secretion of HBsAg and HBeAg in HCC cells. (a) HepG2 and (b) Huh-7 cells transfected with pHBV1.2 plasmid and added with a gradient concentration of AZD8055 or DMSO at 6 h post-transfection. After 48 h, the expressions of HBsAg and HBeAg were detected from the collected supernatant using the ELISA method. Cell viability was assessed using the CCK-8 assay. The values represent the ratio between the treatment and control groups. One-way ANOVA was used, and "ns" indicates no significant effect.

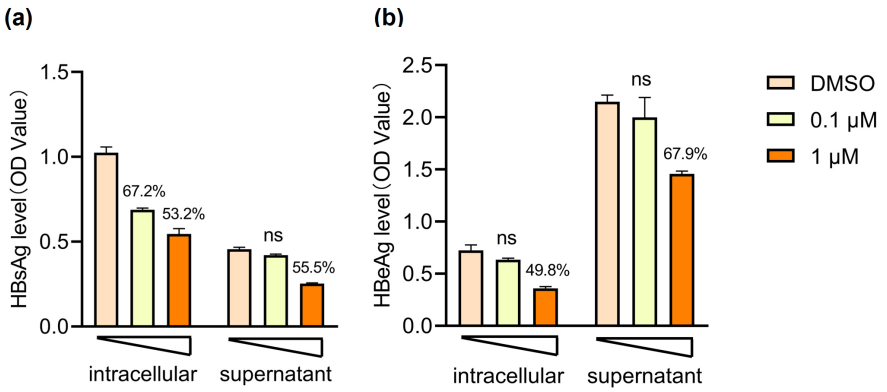

Fig. 2 AZD8055 inhibiting the intracellular expression of HBsAg and HBeAg. (a and b) HepG2 cells transfected with pHBV1.2 plasmid and administered with varying concentrations of AZD8055 or DMSO at 6 h post-transfection. After 48 h, the expressions of HBsAg and HBeAg in lysates of freeze-thaw lysed cells and the supernatants were detected using the ELISA method. The values represent the ratio between the treatment and control groups. One-way ANOVA was used, and "ns" indicates no significant effect.

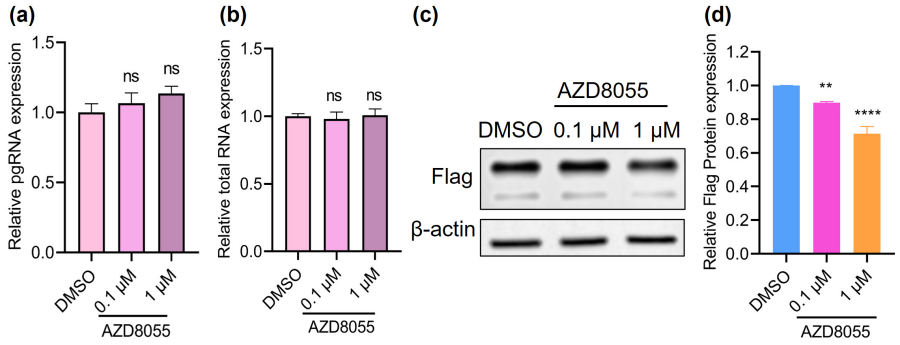

Fig. 3 AZD8055 inhibiting HBsAg expression via post-transcriptional regulation. (a and b) HepG2 cells transfected with the pHBV1.2 plasmid and treated with a gradient concentration of AZD8055, with DMSO as the control. After 48 h, harvested cells underwent RNA extraction, reverse transcription, and RT-qPCR to detect pgRNA and total RNA expression. (c) HepG2 cells transfected with the Flag-HBsAg plasmid and treated with a gradient concentration of AZD8055, with DMSO as the control. After 48 h, harvested cells underwent protein extraction and Western blot analysis using a Flag antibody, with β-actin as the loading control. (d) The quantitative analysis of Flag protein expression. One-way ANOVA was used. ** $p < 0.01$ and **** $p < 0.0001$. "ns" indicates no significant effect.

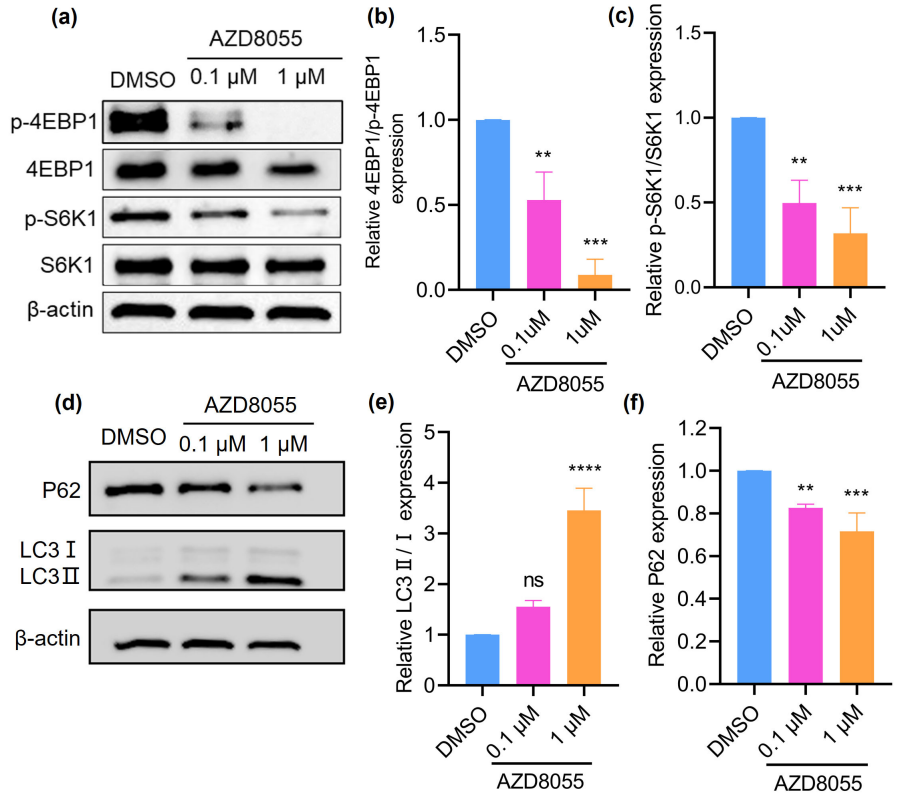

Fig. 4 AZD8055 inhibiting the mTOR pathway and activating autophagy flux. (a) HepG2 cells transfected with the pHBV1.2 plasmid and treated with a gradient concentration of AZD8055, with DMSO as the control. After 48 h, harvested cells underwent protein extraction and Western blot analysis using antibodies against P-4EBP1, 4EBP1, p-S6K1, and S6K1, with β-actin as the loading control. (b and c) Quantitative analysis of the expression of P-4EBP1/4EBP1, P-S6K1/S6K1 proteins. (d) Cell treatment as in panel A, with autophagy-related antibodies P62 and LC3 for Western blot analysis, with β-actin as the loading control. (e and f) Quantitative analysis of LC3II/LC3I and P62 protein expression. One-way ANOVA was used. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. "ns" indicates no significant effect.

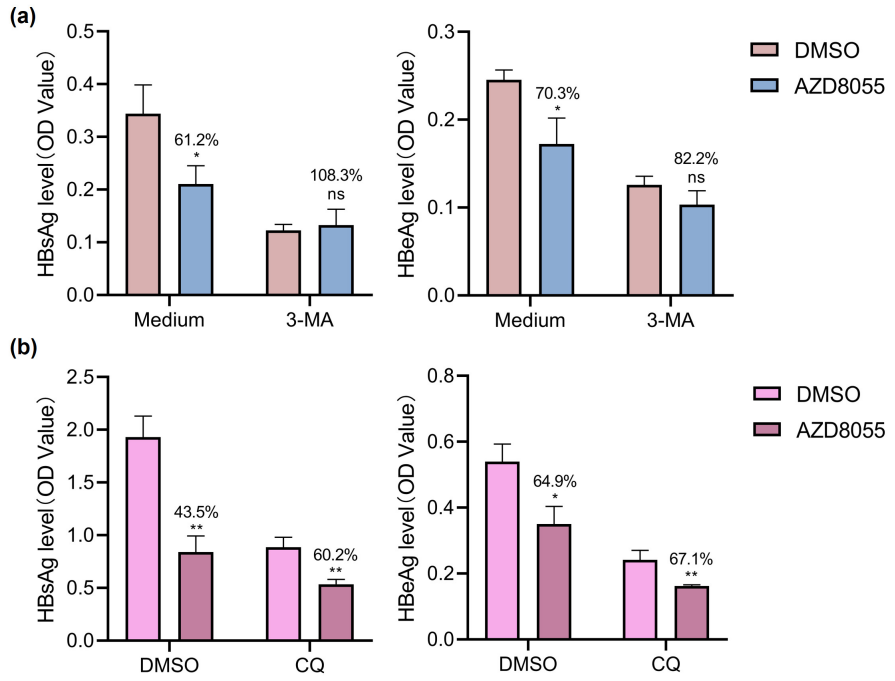

Fig. 5 Autophagy inhibitors 3-MA and CQ blocking the inhibitory effect of AZD8055 on HBsAg expression. (a and b) HepG2 cells transfected with the pHBV1.2 plasmid and treated with AZD8055, along with DMSO, and concurrent treatment with 5 mM 3-MA or 10 μM CQ, with medium or DMSO as the control. After 48 h, expressions of HBsAg and HBeAg were detected from the collected supernatant by ELISA. Data are means ± SD. The values represent the ratio between the treatment and control groups. Statistical significance in (a) and (b) is determined by a two-sided unpaired *t*-test. * $p < 0.05$, ** $p < 0.01$, and "ns" indicates no significant effect.

that directly inhibit HBsAg secretion and production, are currently under exploration [16].

In this study, we have demonstrated that AZD8055, an mTOR inhibitor, effectively reduces HBV antigen secretion in hepatocellular carcinoma cells transfected with the pHBV1.2 plasmid. This suggests that AZD8055 could serve as a potent therapeutic strategy for HBV infection by leveraging autophagy pathways to decrease viral load and pathogenicity without impacting cell viability. This finding is significant as it confirms the role of autophagy in the degradation of viral proteins, as evidenced by our use of autophagy inhibitors like 3-MA and CQ.

Autophagy plays a complex role in the replication of HBV [17]. HBV itself can activate early autophagy but does not increase autophagic degradation, thereby promoting HBV replication [18]; whereas late autophagy, or autophagic degradation, can lead to the degradation of HBV-related proteins, thus inhibiting HBV replication. In our study, it is found that the use of autophagy inhibitors, both 3-MA and CQ, significantly suppresses the expression of HBsAg. Moreover, we observed that if the autophagy pathway is blocked, compared to DMSO, the effect of AZD8055 in inhibiting HBsAg and HBeAg disappears. This indicates that the action of AZD8055 in suppressing HBsAg and HBeAg is dependent on the autophagy pathway. As an early autophagy inhibitor, 3-MA treatment can suppress HBV replication, hence the significant suppression of HBsAg by 3-MA is consistent with literature reports. However, there is a puzzle; according to our view, the autophagy degradation inhibitor CQ should promote the expression of HBsAg [19], but our results found that CQ itself did not significantly enhance the secretion of HBsAg, suggesting that in addition to affecting autophagy, CQ may regulate HBsAg expression through other pathways.

The PI3K/Akt/mTOR pathway and autophagy are critically implicated in the pathogenesis and progression of various cancers [20–22], making them compelling targets in oncological research. Given the role of AZD8055 in inhibiting mTOR, a pathway pivotal for both autophagy and cancer cell survival, its use in HBV-associated HCC could be doubly beneficial. By inhibiting mTOR, AZD8055 may not only suppress the oncogenic signaling conducive to HCC progression but also modify the autophagic flux to reduce the stability and secretion of HBV proteins, thereby potentially reducing viral load and oncogenic drive.

Although these results are promising, their trans-

lation from cell culture models to clinical applications requires further validation. The variable effects of AZD8055 on cell viability between different cell lines, such as HepG2 and Huh-7, call for a comprehensive examination of cell-type specific responses and potential off-target effects. This study paves the way for the development of innovative therapeutic strategies targeting mTOR and autophagy pathways, offering potentially more effective and less resistance-prone options for HBV treatment. Continued research is essential to fully understand these findings and to optimize the use of mTOR inhibitors in treating HBV and potentially other viral infections.

CONCLUSION

Our findings indicate that AZD8055 significantly lowers both the secretion and intracellular concentrations of HBsAg and HBeAg by inhibiting the mTOR pathway and enhancing autophagy. This underscores the potential of AZD8055 as a promising new strategy for treating HBV infection.

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REFERENCES

1. Cui F, Blach S, Manzengo MC, Gonzalez MA, Sabry AA, Mozalevskis A, Seguy N, Rewari BB, et al (2023) Global reporting of progress towards elimination of hepatitis B and hepatitis C. *Lancet Gastroenterol Hepatol* **8**, 332–342.
2. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, et al (2012) Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife* **1**, e00049.
3. Gomez-Moreno A, Ploss A (2024) Mechanisms of hepatitis B virus cccDNA and minichromosome formation and HBV gene transcription. *Viruses* **16**, 609.
4. Ye J, Chen J (2021) Interferon and hepatitis B: Current and future perspectives. *Front Immunol* **12**, 733364.
5. Pierra Rouviere C, Dousson CB, Tavis JE (2020) HBV replication inhibitors. *Antiviral Res* **179**, 104815.
6. Zou Z, Tao T, Li H, Zhu X (2020) mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. *Cell Biosci* **10**, 31.
7. Panwar V, Singh A, Bhatt M, Tonk RK, Azizov S, Raza AS, Sengupta S, Kumar D, et al (2023) Multifaceted role of mTOR (mammalian target of rapamycin) signaling pathway in human health and disease. *Signal Transduct Target Ther* **8**, 375.
8. Wang X, Wei Z, Jiang Y, Meng Z, Lu M (2021) mTOR signaling: The interface linking cellular metabolism and hepatitis B virus replication. *Virol Sin* **36**, 1303–1314.
9. Shlomai A, Paran N, Shaul Y (2006) PGC-1alpha controls hepatitis B virus through nutritional signals. *Proc Natl Acad Sci USA* **103**, 16003–16008.
10. Reese V, Ondracek C, Rushing C, Li L, Oropeza CE, McLachlan A (2011) Multiple nuclear receptors may regulate hepatitis B virus biosynthesis during development. *Int J Biochem Cell Biol* **43**, 230–237.
11. Wang X, Wei Z, Lan T, He Y, Cheng B, Li R, Chen H, Li F, et al (2022) CCDC88A/GIV promotes HBV replication and progeny secretion via enhancing endosomal trafficking and blocking autophagic degradation. *Autophagy* **18**, 357–374.
12. Zhang Y, Li L, Cheng ST, Qin YP, He X, Li F, Wu DQ, Ren F, et al (2022) Rapamycin inhibits hepatitis B virus covalently closed circular DNA transcription by enhancing the ubiquitination of HBx. *Front Microbiol* **13**, 850087.
13. Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE, Vincent JP, Ellston R, et al (2010) AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with *in vitro* and *in vivo* antitumor activity. *Cancer Res* **70**, 288–298.
14. Kawata T, Tada K, Kobayashi M, Sakamoto T, Takiuchi Y, Iwai F, Sakurada M, Hishizawa M, et al (2018) Dual inhibition of the mTORC1 and mTORC2 signaling pathways is a promising therapeutic target for adult T-cell leukemia. *Cancer Sci* **109**, 103–111.
15. Moini M, Fung S (2022) HBsAg loss as a treatment endpoint for chronic HBV infection: HBV cure. *Viruses* **14**, 657.
16. Abdelwahed AH, Heineman BD, Wu GY (2023) Novel approaches to inhibition of HBsAg expression from cccDNA and chromosomal integrants: A review. *J Clin Transl Hepatol* **11**, 1485–1497.
17. Lin Y, Zhao Z, Huang A, Lu M (2020) Interplay between cellular autophagy and hepatitis B virus replication: A systematic review. *Cells* **9**, 2101.
18. Sir D, Tian Y, Chen WL, Ann DK, Yen TS, Ou JH (2010) The early autophagic pathway is activated by hepatitis B virus and required for viral DNA replication. *Proc Natl Acad Sci USA* **107**, 4383–43888.
19. Lin Y, Wu C, Wang X, Liu S, Zhao K, Kemper T, Yu H, Li M, et al (2020) Glucosamine promotes hepatitis B virus replication through its dual effects in suppressing autophagic degradation and inhibiting MTORC1 signaling. *Autophagy* **16**, 548–561.
20. Wisetsathorn S, Tantithavorn V, Hirankarn N, Tangkijvanich P, Saethang T, Kimkong I (2017) Gene polymorphisms of autophagy machinery and the risk of hepatitis B virus-related hepatocellular carcinoma in a Thai population. *ScienceAsia* **43**, 362–368.
21. Sun M, Guo HJ (2023) Role of autophagy-mediated miR-18a in breast cancer progression. *ScienceAsia* **49**, 177–183.
22. Tao R, Wang F, Feng XK, Yang WD (2022) miR-10a-5p inhibits the migration and invasion of human oral carcinoma cells by targeting PIK3CA through PI3K/AKT/mTOR pathway. *ScienceAsia* **48**, 538–544.