Investigation of Novel Endocannabinoid Enzyme Inhibitor ABHD2

LIBERTY UNIVERSITY

Adam Putney, Richard Tuttle, Ersilia Mirabelli, PhD

Abstract and/or Background

Hepatitis B virus (HBV) infection presents a major global health concern, affecting millions of individuals each year. *In-vitro* studies have demonstrated the role of serine hydrolase, specifically α/β hydrolase domain-containing 2 (ABHD2) in hepatitis B viral propagation. The downregulation of ABHD2 via antisense oligonucleotides (ASODNs) decreased HBV mRNA and protein levels, implicating the role of ABHD2 in HBV replication and expression (1). However, the downregulation of ABHD2 was not demonstrated to effectively prevent HBV propagation in further studies (1). In addition, the mechanism of reducing HBV replication and expression via ABHD2 downregulation is not fully understood.

Endocannabinoid enzyme inhibitors, often associated with pain and inflammation, may inhibit serine hydrolase, specifically ABHD2, and ultimately, reduce HBV propagation (2). Previous studies in HepG2.2.15 cells have described a positive correlation between IL-23 expression and HBV infection and progression into hepatocellular carcinoma (HCC) (3). Similarly, reactive oxygen species (ROS) has been associated with an increase in HBV capsid assembly and HBV DNA in HepG2.2.15 cells (4). Through quantifying ROS and IL-23, we seek to determine the efficacy of such inhibitors on HBV infection and propagation *in-vitro* using in HepG2.2.15 cells. This research suggests the possibility of novel drug therapy for regulating HBV propagation through ABHD2 inhibition via endocannabinoid enzyme inhibitors.

Introduction and/or Research Question

This research aims to investigate the mechanism and effects of blocking serine hydrolase via endocannabinoid enzyme inhibitors in hepatocytes that express the HBV virus, specifically HepG2.2.15 cells overexpressing ABHD2. The efficacy of reducing HBV replication by inhibiting ABHD2 in comparison to downregulating ABHD2 demonstrates potential for antiviral treatments for HBV. This research also provides a review of the potential of endocannabinoid enzyme inhibitors in regulating HBV propagation through ABHD2 inhibition.

Methods

HepG2.2.15 cells overexpressing ABHD2 will be cultured appropriately in a sterile environment. HepG2.2.15 cells were selected as commonly-used model for HBV-associated liver disease as it provides stable HBV expression (5). HepG2.2.15 cells overexpressing ABHD2 will be transfected with endocannabinoid enzyme ABHD2 inhibitor (Figure).

The effects and mechanism of ABHD2 inhibition will be evaluated using IL-23 and ROS ELISA kits in HepG2.2.15. Detection of IL-23 has demonstrated a positive correlation with HBV infection as a proinflammatory cytokine. Detection of ROS has demonstrated a positive correlation with HBV capsid assembly and HBV DNA replication.

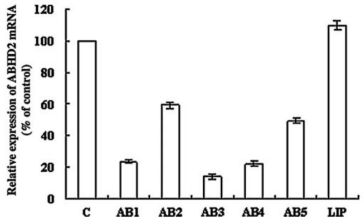

Figure 1. Expression of ABHD2 mRNA via Antisense Oligonucleotides (ASODNs). The graph demonstrates decreased ABHD2 mRNA in the presence of 0.8 μM ADSONs (1).

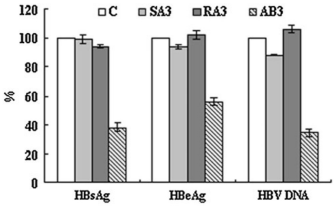

Figure 2. Reduction of HBV Replication and Expression via ABHD2 Downregulation via AB3. The graph demonstrates decreased HBV DNA and HB antigen binding via ABHD2 downregulation (1).

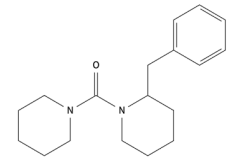

Figure 1. Structure of Standard Endocannabinoid ABHD2 Inhibitor

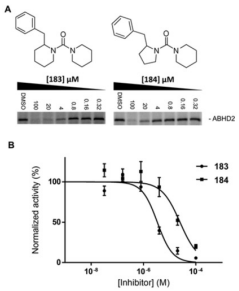

Figure 2. Structure and Activity for Standard ABHD2 Inhibitors. (A) Structures of lead ABHD2 inhibitors and concentration-dependent effect for ABHD2 inhibition. (B) Dose-response relationship of endocannabinoid ABHD2 inhibitors (2).

Results and/or Conclusion

1. Transfection with various ADSONs in HepG2.2.15 cells resulted in significant downregulation of serine hydrolase ABHD2 (Figure 1) (1).
2. Downregulation of ABDH2 via AB3 (ADSON) demonstrated decreased HBV replication and expression via reduced HBV DNA and HB antigens, implicating the essential role of ABHD2 in HBV propagation (Figure 2) (1). Further studies did not demonstrate the efficacy of ABHD2 downregulation in HBV propagation.
3. ABHD2 inhibition, similar to downregulation, resulted in the decreased ABHD2 activity, demonstrating effective inhibition.

Future Work

1. HepG2.2.15 cells overexpressing ABHD2 will be cultured and transfected with endocannabinoid enzyme ABHD2 inhibitor.
2. IL-23 assays will be performed to detect HBV propagation via a positive correlation between IL-23 expression and HBV propagation.
3. ROS detection will be performed to determine HBV capsid assembly and HBV DNA replication following ABHD2 inhibition.

References and/or Acknowledgments

1. Ding X, Yang J, Wang S. Antisense oligonucleotides targeting abhydrolase domain containing 2 block human hepatitis B virus propagation. Oligonucleotides. 2011;21(2):77-84.
2. Baggelaar MP, den Dulk H, Florea BI, Fazio D, Bernabò N, Raspa M, et al. ABHD2 Inhibitor Identified by Activity-Based Protein Profiling Reduces Acrosome Reaction. ACS Chem Biol. 2019 Oct 18;14(10):2295-304.
3. Jiang Q, Sun Y, Guo Z, et al (2018) IL-23 enhances the malignant properties of hepatoma cells by attenuation of HNF4α. Oncotarget 9:28309–28321.
4. Kim YS, Seo HW, Jung G (2015) Reactive oxygen species promote heat shock protein 90-mediated HBV capsid assembly. Biochemical and Biophysical Research Communications 457:328–333.
5. https://www.sigmaaldrich.com/US/en/product/mm/scc249 (Accessed 18 March 2024).
6. Tang C, Chen S, Qian H, Huang W (2012) Interleukin-23: as a drug target for autoimmune inflammatory diseases. Immunology 135:112–124. https://doi.org/10.1111/j.1365-2567.2011.03522.x

We thank Dr. Fulp and Dr. Gilley for their advice and any materials they provided us. We thank the Department of Biology and Chemistry of Liberty University and Center for Research and Scholarship (CRS) at Liberty University for their financial support of this project.