Hijacking HBV Pol to Induce Apoptosis Specifically in Infected Hepatocytes In Vivo: A Novel Approach for Potential Treatment or Cure

Serhat Gümrükçü, Tung X Nguyen, Michael Bobardt, Joseph Kuo, Phillip Musikanth, Philippe Gallay
Disclosure

The following experiments were carried out by employees of Enochian Biosciences.
Introduction HBV

- Despite an effective vaccine, approximately 5 percent of the world’s population chronically carry hepatitis B virus (HBV) and nearly 1 million people die each year.

- Several promising avenues for treatment have been pursued including depleting the pool of covalently closed circular (ccc)DNA and inhibiting HBV polymerase (HBV pol), which is essential both for its reverse transcription and packaging functions.

- In this study, rather than trying to inhibit the HBV life cycle, we utilize the virus and cellular machinery to kill the infected cells as a potential cure.
Novel Mechanism Of Action

- AAV particles were packaged with a novel and proprietary vector construct that expresses a non-functional, non-coding (nc)RNA, HBV “Hijack RNA”, under a strong promoter.

- Open reading frame (ORF) of HBV Hijack RNA is derived from the negative strand of human caspase-9 (casp-9) coding region, and it is flanked between the HBV epsilon signals.

- HBV epsilon signal is the recognition sequence that is specific to HBV pol. The reverse transcriptase domain of HBV pol (HBV pol/RT) recognizes the Hijack RNA through these sequences and reversely transcribes it into a double stranded (ds)DNA that codes for casp-9, driven by a strong promoter.
Novel Mechanism Of Action

HBV epsilon signal is the recognition sequence that is specific to HBV pol. The reverse transcriptase domain of HBV pol (HBV pol/RT) recognizes the Hijack RNA through these sequences and reversely transcribes it into a double stranded (ds)DNA that codes for casp-9, driven by a strong promoter (Fig. 3).
Novel Mechanism Of Action

MECHANISM OF ACTION – HYPOTHESIZED MODEL. A: Delivery of the “Hijack RNA” by AAV to HBV-infected hepatocyte induces apoptosis through overexpression of casp-9 utilizing HBV pol, which engages and reversely transcribes the designed Hijack RNA; B: In the absence of HBV pol in the uninfected hepatocytes, the overexpressed Hijack RNA will undergo degradation, and C: Treatment with HBV pol /RT inhibitors or caspase inhibitors blocks either reverse transcription of the Hijack RNA or activation of casp-9, both of which are needed to trigger the infected cell death through apoptosis.
Methods

- Expression of casp-9 were examined in AAV2-treated HepG2 and the HBV-infected HepAD39 cell lines.
- HBV-infected and uninfected hepatoma cell lines and primary human hepatocytes (PHH) were treated with AAV2 particles expressing Hijack RNA “test AAV” or green fluorescent protein (GFP).
- To validate the mechanism of action, HBV-infected HepAD38 cells were treated with the test AAV in the presence or absence of RT inhibitors tenofovir or entecavir, casp-9-specific inhibitor Z-LEHD-FMK or pan-caspase inhibitor Z-VAD-FMK. Cell viability and proliferation were evaluated by Chemometec NC-200 and FACS analysis using PI/Annexin V and TUNEL assays daily.
HBV-transgenic mice, which constitutively express HBV from kidney and liver cells, received intraperitoneal injections with $10^{11}$ AAV8 particles packaged with Hijack RNA under strong EF1a promoter (DRS1-AAV8) or liver-specific TBG promoter (DRS2-AAV8), or GFP expressing mock/control vector. Day 14 post-injection, liver and kidney tissues were evaluated. Weekly liver enzymatic activities (ALT and AST) were monitored.
Finding 1: Test vector selectively increased casp-9 levels in HBV infected cells

- There was a 254% increase in casp-9 levels in the treated HBV-infected, but not uninfected, cells.
- Casp-9 inhibitor salvaged the Hijack RNA-mediated infected cell death as demonstrated by expansion of viable cells by 10-20%.
Finding 2: HBV Hijack RNA selectively kills HBV-infected or HBV pol expressing cells

Finding 2: HBV Hijack RNA selectively kills HBV-infected or producing cells

- Mean cell death in a variety of HBV-producing cells was 92% (ranging 88.6% to 95.8%), by day 4.
- No significant cell death was observed in uninfected cells.
- RT and pan-caspase inhibitors individually prevented cell death in AAV-treated infected cells.
Finding 3: Mouse liver demonstrated Casp-9 overexpression in HBV-infected hepatocytes treated with Hijack RNA AAV

- Casp-9 IHC staining showed extensive casp-9 overexpression in DRS1-AAV and DRS2-AAV treated, but not in GFP-AAV treated or untreated mice liver tissue.

A. DRS1-AAV  
B. DRS2-AAV  
C. UNTREATED  
D. GFP-AAV

CYTOLOGY ANALYSIS revealed high level of casp-9 (IHC brown stain) in HBV-infected hepatocytes from mice treated with Hijack RNA AAV (A and B) but not in untreated (C) or GFP-AAV control vector treated mice (D)
Finding 4: Hijack RNA AAV induced extensive neutrophil infiltration indicating HBV-infected hepatocyte damage

Diffuse neutrophil infiltration indicating an inflammatory reaction to HBV-infected hepatocytes in mice treated with Hijack RNA AAV.

Untreated mice or mice treated with GFP AAV control vector exhibited no evidence of inflammation.

Deep purple staining demarcates neutrophil infiltration in untreated (A), GFP-AAV control vector-treated (B) and Hijack RNA AAV vector-treated (C,D) mice.
Finding 5: Increased liver enzymes in mice treated with HBV Hijack RNA AAV indicate hepatocellular damage

- AST levels increased 2.2 fold and 1.8 fold in mice treated with AAV-DRS1 and AAV-DRS2, respectively compared to GFP-AAV treated and untreated mice at week 4.

- ALT levels increased 1.4 fold in both treatment groups compared to GFP-AAV treated and untreated mice.
DISCUSSIONS

- Hijacking HBV pol to express casp-9 induces significant cell death in HBV-infected but not in HBV-uninfected cells \textit{in vitro}.

- Reverse transcriptase and caspase inhibition abrogated cell death, validating the hypothesized mechanism of action.

- Data from \textit{in vivo} studies demonstrate that HBV Hijack RNA induces apoptosis of HBV-infected hepatocytes resulting in inflammation and increased liver enzymes.

- However, transgenic mouse models are inherently limited to study the potential for HBV cure since it is impossible to eradicate HBV in animals that constitutively expresses the HBV genome in every hepatocyte.
CONCLUSIONS

- These results demonstrate a novel mechanism of action for a potential cure for HBV infection.
- Additional in vitro and in vivo studies are in progress.
Acknowledgments

Enochian BioSciences Inc.
Los Angeles, CA

• Tung Nguyen

Seraph Research Institute
Los Angeles, CA

Serhat Gümrükçü MD PhD
Phillip Musikanth MD
Tung Nguyen

Scripps Research Institute
La Jolla, CA

• Philippe Gallay PhD
• Joseph Kuo PhD
• Michael Bobardt