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

Serhat Gümrukçü1, Tung X Nguyen1,2, Michael Bobardt1, Joseph Kuo3, Phillip Musikanth1, Philippe Gallay3
1Seraph Research Institute, Los Angeles, CA; 2Enochian Biosciences, Los Angeles, CA; Department of Immunology & Microbiology, The Scripps Research Institute, La Jolla, CA

BACKGROUND

- Despite an effective vaccine, approximately 5 percent of the world’s population chronically carries 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.

MECHANISM OF ACTION

- AVV 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 (Fig. 1).
- 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 opsin signals (Fig. 2).
- 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 reversibly transcribes it into a double stranded (ds)DNA that codes for casp-9, driven by a strong promoter (Fig. 3).
- The dsDNA engages with host polymerases to overexpress casp-9 and induce apoptosis of the infected cell (Fig. 4A).
- In an uninfected cell, without the presence of HBV pol, Hijack RNA would be non-functional and be degraded (Fig. 4B).

METHODS

- HBV-transgenic mice, which constitutively express HBV from kidney and liver cells, received intraperitoneal injections with 1010 AVV particles packaged with Hijack RNA under strong EF1a promoter (DRS1-AVH) or liver-specific Tsk promoter (DRS2-AVH), or GFP expressing mock/control vector (Fig. 5). Day 14 post-injection, liver and kidney tissues were evaluated. Weekly liver enzymatic activities (ALT and AST) were monitored.

RESULTS

Finding 3: Mouse liver demonstrated Casp-9 overexpression in HBV-infected hepatocytes treated with Hijack RNA AVV
- Casp-9 IHC staining showed extensive casp-9 overexpression in DRS1-AVH and DRS2-AVH treated, but not in GFP-AVH treated or untreated mice liver tissue.

Finding 4: Hijack RNA AVV 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.
- Untreated mice or mice treated with GFP AVH control vector exhibited no evidence of infiltration.

Finding 5: Increased liver enzymes in mice treated with HBV Hijack RNA AVV indicate hepatocellular damage
- ALT levels increased 2.2 fold and 1.3 fold in mice treated with AVH-DRS1 and AVH-DRS2, respectively compared to GFP-AVH treated and untreated mice at week 6.
- ALT levels increased 1.4 fold in both treatment groups compared to GFP-AVH treated and untreated mice.

DISCUSSION

- Hijacking HBV pol to express casp-9 induces significant cell death in HBV-infected but not in HBV-uninfected cells in vitro.
- Reverse transcriptase and caspase inhibition abrogated cell death, validating the hypothesized mechanism of action.
- Data from 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.