Inarigivir is a novel selective inhibitor of the HBV replicase complex in vitro

Danni Colledge1, Kathy Jackson1, Vitina Sozzi1, Xin Li1, Michael Beard2, Nicholas Eyre3, Junjie Zhang3, Haitao Guo3, Nezam Afshahi4, Radhakrishnan Iyer5, Stephen Locarnini1

1 Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia. 2 The University of Adelaide, Adelaide, Australia. 3 Indiana University School of Medicine, Indianapolis, IN USA, 4 Spring Bank Pharmaceuticals, Hopkinton, MA, USA

Introduction

- Inarigivir (SB 9200) is a linear dinucleotide antiviral drug that has been shown to act as an RIG-I agonist to activate cellular innate immune responses.
- Clinical trials in HCV patients have confirmed the innate activation by Inarigivir for antiviral activity and in HBV patients have demonstrated significant reduction in HBV DNA and RNA.
- To further determine the antiviral mechanism of Inarigivir in hepatocyte-derived cells, we set out to characterize the antiviral effect of Inarigivir in HBV transiently transfected HepG2 cells and in the inducible HBV stable cell lines including HepAD38 and HepDES19 cells.

Methods

- The antiviral effect of Inarigivir to HBV was investigated in transiently transfected HepG2 cells and stably transfected HepG2AD38 and HepDES19 cells.
- HBV protein, DNA and RNA replication intermediates were analysed by Southern, Western and Northern blotting and endogenous polymerase assay and real time quantitative PCR.

Results

1. Antiviral effect of SB 9200 in HBV Transiently Transfected HepG2 Cells

HepG2 cells were transiently transfected with HBV 1.3. Results indicate that SB 9200 did not affect HBV RNA packaging but blocked capsid-dependent DNA replication.

2. Antiviral effect of SB 9200 in HBV Stably Transfected Cell Lines

In stable cell lines expressing HBV, again SB 9200 did not affect HBV RNA packaging and confirmed lack of effect of pgRNA packaging yet blocked HBV DNA replication.

3. Assessment of antiviral effect of SB9200 compared to other HBV inhibitors

BAY41-109 affected core production and capsid assembly, AT-61 affected pgRNA encapsidation and packaging and Lamivudine inhibited reverse transcription. SB 9200 had no effect on core production or assembly.

4. Effect of SB 9200 on HBV polymerase

SB 9200 exhibited dose-dependent inhibition of the HBV-endogenous DNA polymerase, therefore, unlike nucleoside analogues, is not a chain terminator.

5. Effect of SB 9200 on the innate signalling pathway

SB 9200 is not a strong stimulator of innate signalling in hepatoma cells.

Conclusions

- Inarigivir (SB 9200) exhibits potent anti-HBV activity in cell cultures primarily through blocking DNA replication without affecting viral nucleocapsid assembly, indicating a novel mechanism of action.
- In addition its innate immunomodulatory function in vivo, Inarigivir possesses direct antiviral activity against HBV replication in vitro, which is highlighted in hepatoma cells refractory to Inarigivir-induced innate activation.
- The DAA effect of Inarigivir appears to involve inhibition of HBV replication at the level of reverse transcription and/or blocking priming or subsequent primer translocation within the viral nucleocapsid.

Support

Research Support

Fig 1: HBV Life Cycle

Fig 2: Antiviral effect of SB 9200 in HepG2 cells that have been transiently transfected with HBV.

Fig 3: Antiviral effects of SB 9200 in HBV stably transfected cell lines. (A) HepDES19 (tet-) cells, (B) HepAD38 (tet-) cells.

Fig 4: Antiviral effects of SB 9200 in HBV stably transfected HepDES19 cells compared to other known HBV inhibitors. (A) Site of action of known HBV inhibitors and that proposed for SB 9200 which is blocking RT priming and/or primer translocation (X) (C) Chemical structure of Inarigivir.