Introduction

- Inarigivir (SB 9200) is a synthetic dinucleotide antiviral drug that has been shown to act as an RIG-I agonist to activate cellular innate immune responses (Figure 1).
- 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.
- Inarigivir has demonstrated direct antiviral activity (DDA) against HBV in vitro by inhibiting reverse transcription following the packaging of its gRNA into the nucleocapsid (Colledge et al, 2018).
- The aim of this study was to test the in vitro antiviral potency of Inarigivir against a panel of nucleos(t)ide analogue resistant, core protein assembly modulator (CPAM) inhibitor resistant and precore stop codon variants of HBV.

Methods

- The antiviral effect of inarigivir against HBV was investigated in HuH7 cells transiently transfected with replication competent clones known to be resistant to (Zoulim and Locarnini, 2009):
  - Lamivudine (LMV): rT180M/rM204V and rT204I
  - Adeovir (ADV): rT181V/rN236T
  - Entecavir (ETV): rT180M/rT184G/rS202I/rM204V (Quad)
- The antiviral effect of Inarigivir against HBV was also investigated in HuH7 cells transiently transfected with a panel of HBVs with substitutions in the core protein which have been shown to have reduced sensitivity to the CPAM class of inhibitors (Klump et al, 2015):
  - cT33I
  - cT109A
  - cT118F
  - cV124A
  - cY132A
- The effect on the HBsAg negative G1896A stop codon variant was also investigated in HuH7 cells by transfection.
- After 5 days of drug treatment, cells were harvested, core preps made, DNA extracted and Southern blots performed.
- Results were scored as drug sensitive to Inarigivir if a dose-response in antiviral effect on the HBV DNA replicative intermediate single strand (SS) was observed and the inhibition exceeded 20% compared to the untreated control.

Results

1. Antiviral effect of Inarigivir (SB 9200) to HBV variants with known nucleos(t)ide analogue resistance

Fig 2A shows Southern blots of WT and drug resistant HBVs in the presence of SB 9200, BAY 41-4109 and LMV compared to the untreated control (C0MM). Estimate of DNA replication levels were derived from densitometric readings and normalised to 100% against the untreated controls (Figure 2B). In HuH7 cells transiently transfected with replication competent clones of HBV known to be resistant to lamivudine, entecavir or adefovir, Inarigivir retained antiviral activity against all of the drug resistant variants tested. As expected, Lamivudine and Entecavir have reduced sensitivity and/or resistance to these drug resistant variants.

2. Antiviral effect of Inarigivir (SB 9200) to HBV variants with core mutations shown to have reduced sensitivity to HBV core inhibitors

Figure 3A shows Southern blots of WT and HBV replicon yields with a range of core mutations in the presence of SB 9200, BAY 41-4109 and LMV compared to the untreated control (C0MM) as shown in Figure 3A. Estimate of DNA replication levels were derived from densitometric readings and normalised to 100% against the untreated controls (Figure 3B). All variants were replication competent although cT33I variant is only weakly replicative. Southern blot analysis shows that Inarigivir has activity against all of the core variants tested. Even though the Y132A construct was able to produce and secrete HBsAg and HBsAg it was replication incompetent (results not shown).

Conclusions

- These studies indicate that Inarigivir (SB 9200) is active against HBV variants carrying resistance markers against all the nucleos(t)ide analogues approved for treating chronic hepatitis B.
- Furthermore, this study extended these results to include the recently identified CPAM inhibitor resistant variants and HBVs with the precore stop codon G1896A which were all sensitive to Inarigivir.
- Collectively, this data indicates that Inarigivir treatment could be suitable rescue therapy in those patients who have failed nucleos(t)ide analogue therapy due to resistance and also for HBV with resistance markers to the CPAM group, thus positioning Inarigivir as a suitable cornerstone in future hepatitis B treatments.

References


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