The Novel Antiviral Agent Inarigivir Inhibits Both Nucleos(t)ide Analogue and Capsid Assembly Inhibitor Resistant HBV *in vitro*

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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 (DAA) against HBV *in vitro* by inhibiting reverse transcription following the packaging of its pgRNA 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 modifier (CpAM) inhibitor resistant and precore stop codon variants of HBV.

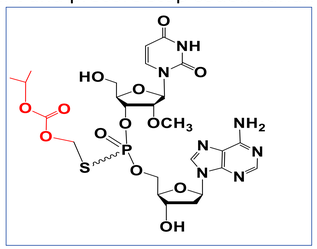


Fig 1: Chemical structure of Inarigivir (SB 9200)

Methods

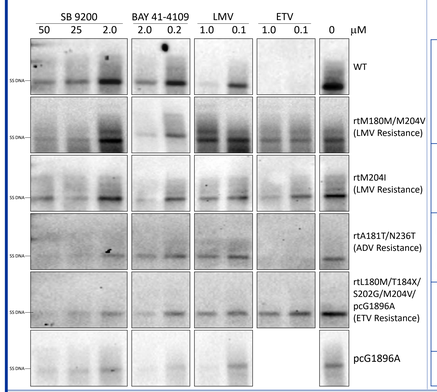
- The antiviral affect of Inarigivir against HBV was investigated in Huh7 cells transiently transfected with replication competent clones known to be resistant to (Zoulim and Locarnini, 2009):
  - Lamivudine (LMV): rtL180M/rtM204V and rtM204I
  - Adefovir (ADV): rtA181V/rtN236T
  - Entecavir (ETV): rtL180M/rtT184G/rtS202I/rtM204V (Quad)
- The antiviral affect 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 (Klumpp *et al*, 2015):
  - cT33I
  - cY109A
  - cT118F
  - cV124A
  - cY132A
- The effect on the HBeAg negative G1896A stop codon variant was also investigated in Huh7 cells by transient 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 (0μM). 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 the drug resistant variants.

2A: Southern blot



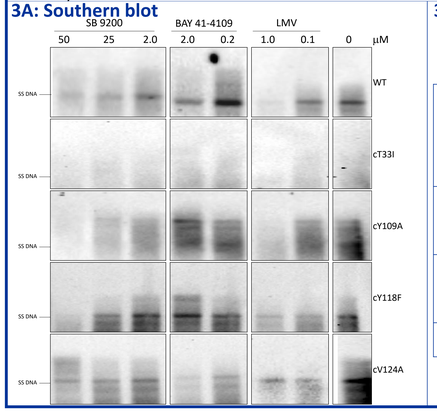
2B: Densitometric analysis

	rtL180M/ M204V	rtM204I	rtA181V/ N236T	PC/Quad	WT	pcG1896A
SB 50μM	45.45	65.23	67.01	45.80	49.58	50.55
SB 25μM	47.29	54.17	68.76	47.97	52.28	52.84
SB 2.0μM	140.94	100.68	85.92	54.90	84.78	112.17
BAY 2.0μM	44.58	33.31	79.62	34.63	41.01	64.82
BAY 0.2μM	106.98	70.23	101.39	61.92	71.24	112.34
LMV 1.0μM	81.74	50.51	89.38	52.84	16.47	8.48
LMV 0.1μM	98.25	72.12	102.31	65.78	55.97	35.32
ETV 1.0μM	37.54	47.87	47.50	59.44	7.43	-
ETV 0.1μM	69.39	71.94	59.47	69.08	12.93	-
Untreated	100.00	100.00	100.00	100.00	100.00	100.00

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

Figure 3A shows Southern blots of WT and HBV replication yields with a range of core mutations in the presence of SB 9200, BAY 41-4109 and LMV compared to the untreated control (0μM) 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 cY132A construct was able to produce and secrete HBsAg and HBeAg it was replication incompetent (results not shown).

3A: Southern blot



3B: Densitometric Analysis

	cT331	cY109A	cY118F	cV124A	WT
SB 50μM	47.85	27.90	19.76	40.57	42.97
SB 25μM	63.63	58.42	76.43	42.08	52.53
SB 2.0μM	59.34	76.38	138.19	55.55	77.51
BAY 2.0μM	63.42	159.56	150.97	58.74	50.43
BAY 0.2μM	74.53	101.35	115.04	61.05	81.30
LMV 1.0μM	7.25	31.02	35.74	17.04	22.80
LMV 0.1μM	70.05	93.72	52.88	37.35	77.02
Untreated	100.00	100.00	100.00	100.00	100.00

3. Proposed site of action of Inarigivir

The DAA effect of Inarigivir has been shown to involve inhibition of HBV replication at the level of reverse transcription and/or blocking priming or subsequent primer translocation within the viral nucleocapsid (Colledge *et al*, 2018) (see Figure 4). Inarigivir is active against both classes of drug resistant mutants; Figure 4 highlights no reduction in antiviral efficacy of Inarigivir due to these variants.

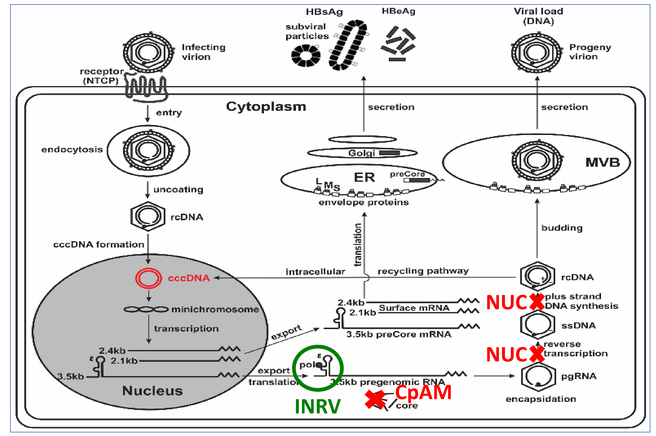


Fig 4: HBV Life Cycle and the site of action of CpAM and nucleoside analogues (NUC). Green circle denotes MOA of Inarigivir (INRV).

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

1. Colledge *et al*. 2018. Inarigivir is a novel selective inhibitor of the HBV replicase complex in vitro. Hepatology 68 (S1): abstract 383.
2. Zoulim and Locarnini. 2009. Hepatitis B virus resistant to nucleos(t)ide analogues. Gastroenterology 137: 1593-1608.
3. Klumpp *et al*. 2015. High-resolution crystal structure of a hepatitis B virus replication inhibitor bound to the viral core protein. PNAS 112 (49): 15196-15201.

Research Support

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