GST-HG141, a novel clinical-stage hepatitis B virus capsid assembly modulator

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Background and Aims

Capsid assembly process, a critical step in the hepatitis B virus (HBV) life cycle, has recently emerged as a key target for the treatment of chronic hepatitis B. The capsid assembly modulators (CAMs) have been validated in the clinic and have a potential for HBV cure. Here we report preclinical characterization of GST-HG141, a novel HBV CAM, including molecular mechanism(s) of its antiviral action and the resistance profile *in vitro*. Currently GST-HG141 is under phase I clinical evaluation.

Methods

A purified, recombinant, c-terminally truncated HBV core protein (aa 1-150) was used in the biochemical quenching assay, size-exclusion chromatography and transmission electron microscopy (TEM), to determine the effects on HBV capsid assembly *in vitro*. Effects on secreted HBV DNA were determined in HepG2.2.15 cells, using qPCR. Western, southern and northern blots were performed to detect HBV capsids, HBV capsid-associated DNA and RNA, respectively. In *vivo* antiviral efficacy was assessed in the AAV-HBV mouse model.

Results

GST-HG141 potently inhibited HBV DNA secretion in HepG2.2.15 cells, with the E_{C50} value of 8.16±3.65 nM. In the transient transfection assays, GST-HG141 retained potent antiviral activity against HBV genotypes A-D (EC_{50} 26-228 nM, Table 1), and against CAM- and nucleos(t)ide-resistant mutants (Table 2). No significant cytotoxicity was observed in eleven mammalian cell lines and primary cells (CC_{50}>50 μM for MRC-5, HEK293, Caki-1, HepG2, MT-4, Colo-205, H1 HeLa, A375, Hep2 and Huh7, for CCRF-CEM CC_{50} value was 38 μM).

Table 1. Inhibition of secreted HBV DNA of different genotypes in transiently-transfected HepG2 cells. The mean EC_{50} values in nM are shown.

HBV isolate (genotype)	AP007263 (A)	HE974371 (A)	AB246345 (C)	AB246346 (C)	N406371 (B)	AB033554 (B)	U95551 (D)
GST-HG141	59	33	26	32	40	47	228
Entecavir	0.25	1.93	1.11	2.25	1.34	1.47	1.00

The antiviral activity of GST-HG141 was HBV-specific, as other representative DNA and RNA (plus- or minus-strand) viruses tested were not inhibited in vitro (not shown).

Table 2. Antiviral activity of GST-HG141 against CAM- and nucleos(t)ide resistance mutants. The EC_{50} fold shift values, relative to WT, are shown.

HBV Mutant	GST-HG141	GLS-4	AT-130	ETV	LAM
WT (U95551)	1	1	1	1	1
Core F23Y	4.8	2	18	1	ND
Core P25G	1.2	13	13	0.8	ND
Core T33N	7	98	49	0.9	ND
Core T109M	23	1.7	3.6	0.4	ND
Core V124F	70	63	1.2	0.5	ND
Pol M204I	0.5	ND	ND	ND	>990
Pol M204I+V173L	1.2	ND	ND	ND	>990
Pol M204I+S202G	0.7	ND	ND	4913	ND
Pol M204I+S202G	1.1	ND	ND	ND	>990
Pol M204I+S202G+ M250V	1	ND	ND	3956	ND

Results (cont.)

In the drug combination studies with GST-HG141, GST-HG131 (novel HBV gene expression inhibitor, see Poster #397), Tenofovir or Entecavir, additive antiviral effects were observed (Fig. 1). The effects on HBV DNA and HBsAg secretion by cultured HepG.2.215 cells was measured in these drug combination studies.

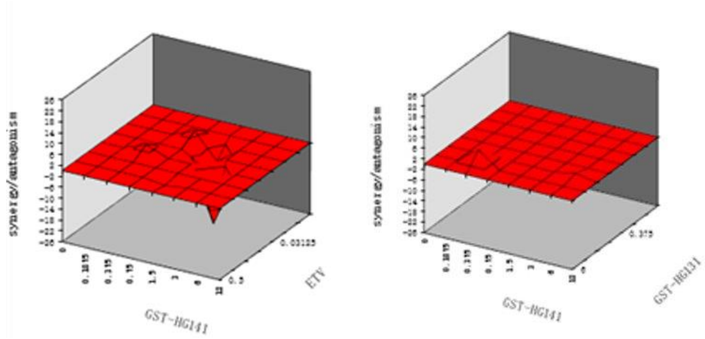

Fig. 1. Representative MacSynergyII plots of drug combination (GST-HG141, Entecavir and GST-HG131) effects on secretion of HBV DNA by cultured HepG.2.2.15 cells.

In the biochemical quenching assay, GST-HG141 induced HBV core protein assembly (EC_{50} = 0.93 ± 0.11 μM). GST-HG141 also induced formation of intact capsids, as was demonstrated by the size-exclusion chromatography experiments (Fig. 2). The TEM studies confirmed that treatment with GST-HG141 did not affect the capsid morphology (Fig. 3).

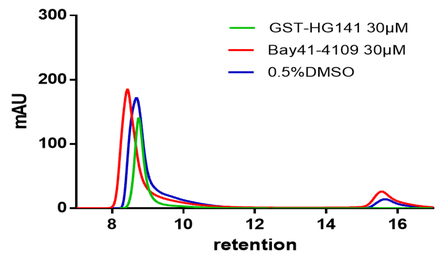

Fig. 2. Size exclusion chromatography of recombinant HBV capsids (aa 1 to 150), incubated with 0.5% DMSO (untreated control), 30 μM GST-HG141 or 30 μM GLS4 for 24 h in the presence of 150 mM NaCl.

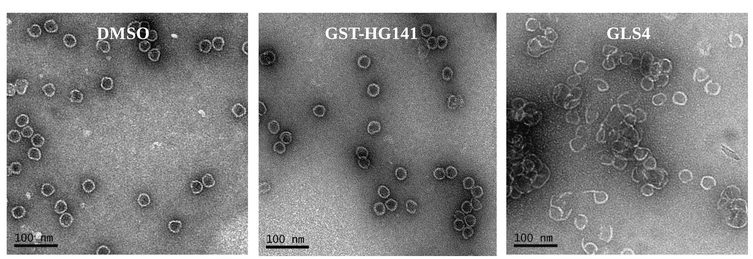

Fig. 3. TEM images of the *in vitro*-assembled purified recombinant HBcAg assembly domain (aa 1-150).

Results (cont.)

In the GST-HG141-treated HepG.2.215 cells, a dose-dependent reduction of capsid-associated HBV DNA and RNA was observed. However, a shift from T=4 towards T=3 capsid formation was not observed, in contrast to other class I CAMs (Fig. 4).

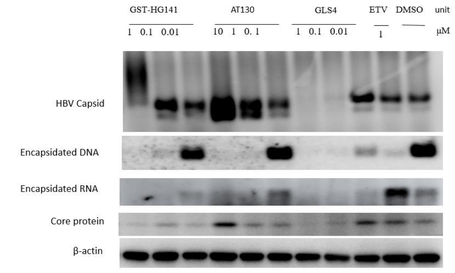

Fig. 4. Effect of GST-HG141 on the capsid-associated HBV DNA and RNA.

In the AAV/HBV model, GST-HG141 demonstrated a robust dose-dependent reduction in serum (~3.0 log_{10}) and liver (0.9 log_{10}) HBV DNA, following 28 days of dosing (Fig. 5). After cessation of treatment, serum HBV DNA levels quickly rebounded. The effect of GST-HG141 on the serum levels of HBV RNA was modest, but statistically significant. After 28 days of treatment with 100 mg/kg GST-HG141, average serum HBV RNA level was reduced by 1.09 log_{10} GE/ml (from 4.74 ± 0.08 to 3.64 ± 0.05 log_{10} GE/ml, p<0.01). GST-HG141 was well-tolerated, no significant effect on animal body weight was observed in any of the animal treatment groups.

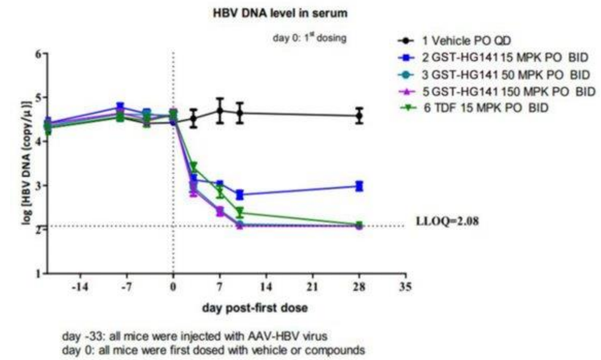

Fig. 5. Effect of GST-HG141 and Tenofovir Dipivoxil (TDF) on the serum HBV DNA levels in the mouse AAV model.

On the 27th day of treatment with 10 mg/kg, the average GST-HG141 trough plasma concentration was 78 nM. Drug absorption peaked 1 hour after administration, the plasma half-life was 2.23 hours, and the $AUC_{0\text{-}inf}$ was 47600 nM.h.

Conclusions

GST-HG141 is a novel, orally-bioavailable HBV CAM. It accelerates HBV capsid assembly *in vitro*, leading to formation of "empty" capsids, devoided of genetic material. However, its mode of action and resistance profile appear to be distinct from other class I CAMs that are currently in development. GST-HG141 has an excellent antiviral potency *in vitro*, as well as efficacy *in vivo*, and is well-tolerated in rodents. Further development of GST-HG141 for chronic HBV infections is warranted. Currently it is undergoing phase I clinical evaluations.