Differential Impact of CAM-E and CAM-A on Hepatitis B Core Protein Phosphorylation States In Vitro

Hannah Vanrusselt[1], Rene Geissler[2], Jordi Verheyen[1], Tse-I Lin[1], Lawrence Blatt[3], Julian Symons[3], Andreas Jekle[3], Megha Patel[2], Gavin Cloherty[2], Yannick Debing[1]

[1] Aligos Belgium BV, Leuven, Belgium; [2] Abbott Laboratories, Abbott Diagnostics Division, Abbott Park, Illinois, United States; [3] Aligos Therapeutics, Inc., South San Francisco, California, United States

Poster 283

BACKGROUND

HBV core protein (HBc) phosphorylation is crucial for hepatitis B virus (HBV) capsid formation, with empty particles showing high HBc phosphorylation and DNA-containing particles showing low levels [1]. Capsid assembly modulators (CAMs) target HBc and modulate the assembly of viral capsids, leading to the formation of empty capsids (CAM-E) or aberrant capsids (CAM-A). This study focused on the differential effects of CAM-E and CAM-A on HBc phosphorylation, quantifying phosphorylated HBc (P-HBcAg) and unphosphorylated HBc (HBcAg) to reveal their roles in viral particle formation.

AIM

Assess the effect of CAMs on HBc phosphorylation in vitro and in vivo.

METHODS

Multiple in vitro assays were employed to evaluate the effects of CAM-E and CAM-A compounds on HBc phosphorylation states. HepAD38 cells and HBV-infected primary human hepatocytes (PHH) were treated with different CAMs and nucleoside analogs in serial dilutions. For the PHH, compound was added together with the HBV inoculum. Medium and cell lysates were collected after 7 days of culture for HepAD38 cells and 12 days of culture for HBV-infected PHH and used for the P-HBcAg and HBcAg chemiluminescent microparticle immunoassays (CMIA) [2]. Specific chemotypes were tested to compare their efficacy and to identify potential mechanistic variations among CAM compounds. Finally, the in vivo effect of CAMs on HBcAg and P-HBcAg was assessed in serum samples from AAV-HBV-transduced mice [3].

RESULTS

CAM-E TREATMENT INCREASES P-HBcAg LEVELS IN HepAD38 CELLS

Non-phosphorylated HBcAg levels, i.e. DNA-containing particles, were strongly reduced in medium and intracellularly upon CAM-E treatment, with an EC_{50} of 38 nM for compound B [4] and 4 nM for ALG-000111 [5] for intracellular HBcAg. These values are in range with historical HBV DNA EC_{50} values for these CAMs. P-HBcAg levels, i.e. empty particles, increased correspondingly in medium and intracellularly. This was seen for both compound B and ALG-000111, both CAM-E compounds.

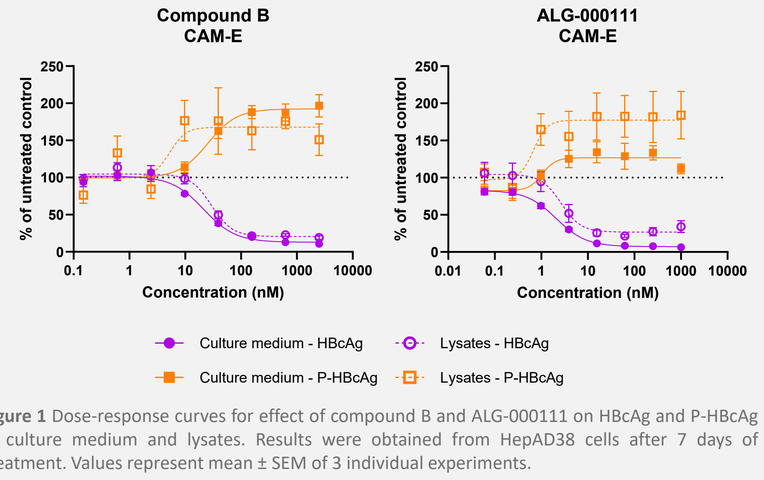

Figure 1 Dose-response curves for effect of compound B and ALG-000111 on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from HepAD38 cells after 7 days of treatment. Values represent mean ± SEM of 3 individual experiments.

CAM-A TREATMENT SHOWS A BIPHASIC PROFILE FOR P-HBcAg LEVELS IN HepAD38

Upon CAM-A treatment, the HBcAg levels were strongly reduced in medium and intracellularly, similar to CAM-E treatment, with an EC_{50} of 33 nM for ALG-006746 [6] and 260 nM for RG7907 [7] for intracellular HBcAg. These EC_{50} values were in line with historical HBV DNA results. P-HBcAg levels were reduced at high concentrations but increased at lower concentrations of CAM-A. Likely, P-HBcAg is trapped in aggregates at high concentrations but soluble and secreted at lower CAM-A concentrations which leads to a biphasic profile. The P-HBcAg EC_{50} values, 282 nM for ALG-006746 and 2182 nM for RG7907, approximate the EC_{50} values for the formation of HBc aggregates from an immunofluorescent staining assay [7].

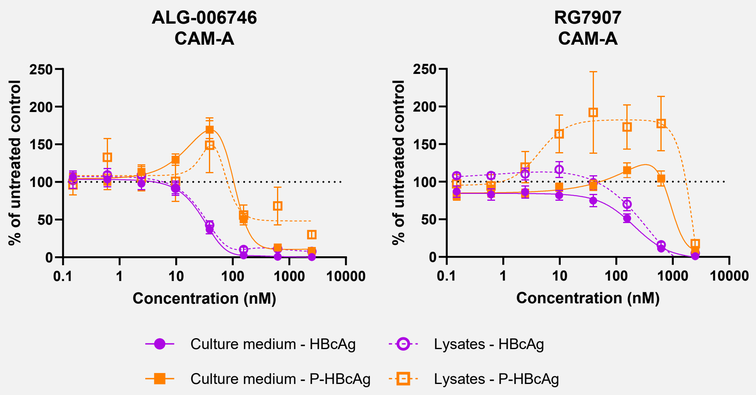

Figure 2 Dose-response curves for effect of ALG-006746 and RG7907 on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from HepAD38 cells after 7 days of treatment. Values represent mean ± SEM of 3 individual experiments.

NUCLEOSIDE ANALOG TREATMENT SHOWS NO EFFECT IN HepAD38 CELLS

No clear effect on HBcAg or P-HBcAg was seen after treatment with nucleoside analogs, entecavir and tenofovir disoproxil fumarate (TDF). No effect was seen either after longer incubation for 14 days.

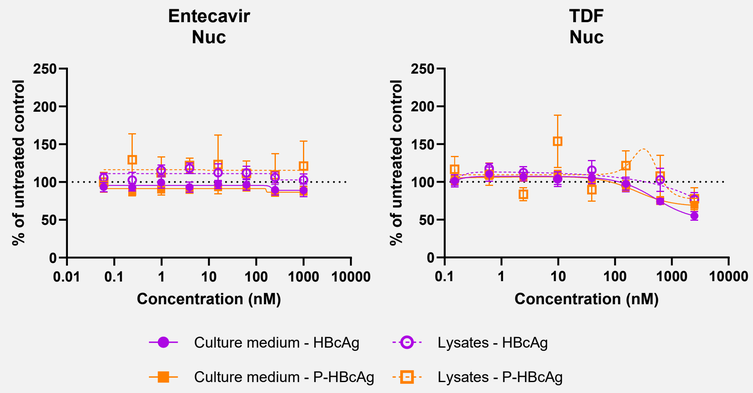

Figure 3 Dose-response curves for effect of entecavir and TDF on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from HepAD38 cells after 7 days of treatment. Values represent mean ± SEM of 3 individual experiments.

CAMs SHOW A SLIGHTLY DIFFERENT EFFECT IN PHH

For all CAMs tested, the HBcAg levels strongly decreased in culture medium and intracellularly, similar to what was seen in HepAD38 cells. The P-HBcAg levels after CAM-E treatment, conversely to what was seen in HepAD38, decreased at high concentrations of the compound and increased at lower concentrations, leading to a biphasic profile. This is likely the consequence of the secondary mechanism of CAMs, blocking cccDNA establishment at the higher concentrations. This was only the case in cell culture lysates. In culture medium, a reduction of P-HBcAg was observed at high concentrations for all compounds.

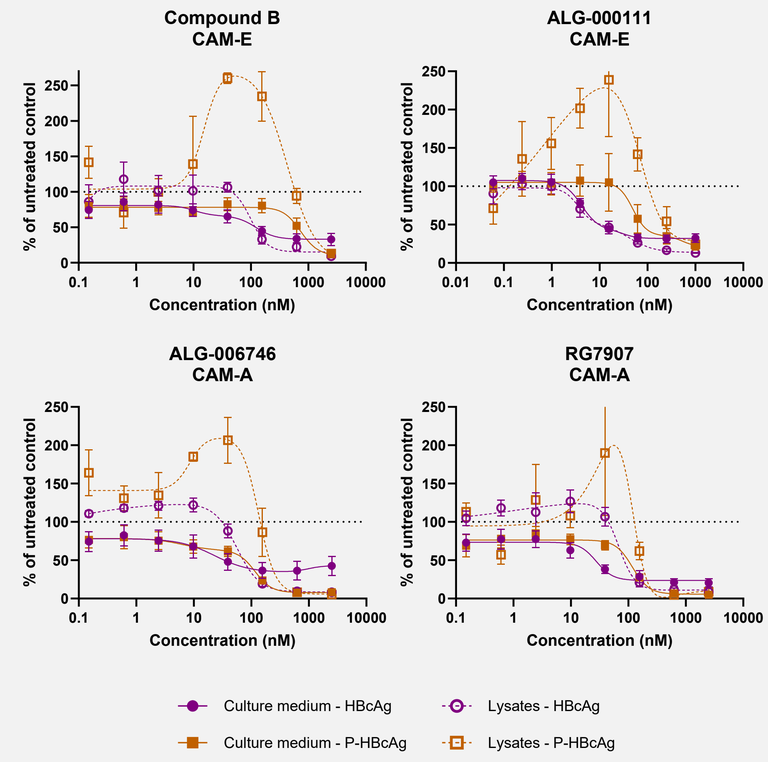

Figure 4 Dose-response curves for effect of compound B, ALG-000111, ALG-006746 and RG7907 on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from PHH after 12 days of treatment. Values represent mean ± SEM of 2 individual experiments.

NUCLEOSIDE ANALOG TREATMENT SHOWS SOME EFFECT IN PHH

Upon treatment with entecavir, a nucleoside analog, both HBcAg and P-HBcAg are slightly reduced at the higher concentrations, both in culture medium and lysates. The relative EC_{50} values are comparable with historical HBV DNA values for entecavir.

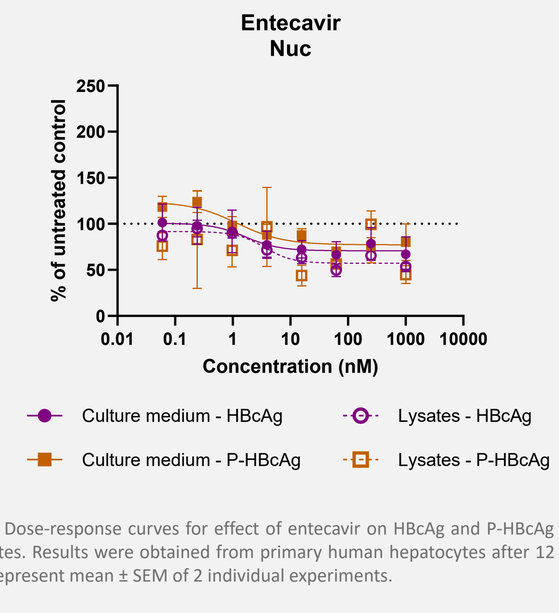

Figure 5 Dose-response curves for effect of entecavir on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from primary human hepatocytes after 12 days of treatment. Values represent mean ± SEM of 2 individual experiments.

CAM TREATMENT IN AAV-HBV MICE

AAV-HBV mice were treated with compound B at 50 mg/kg BID for 42 days and with RG7907 at 20 mg/kg QD for 83 days. Treatment with a CAM-E, compound B, induced a slight reduction of HBcAg levels and a more pronounced reduction of P-HBcAg levels in serum samples. While the CAM-A, RG7907, showed >99% reduction of both HBcAg and P-HBcAg levels in serum samples, likely due to the loss of AAV-HBV-positive hepatocytes after prolonged CAM-A treatment [7].

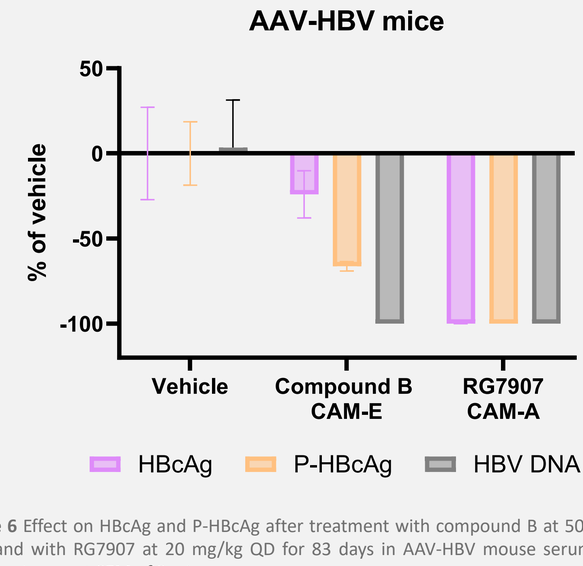

Figure 6 Effect on HBcAg and P-HBcAg after treatment with compound B at 50 mg/kg BID for 42 days and with RG7907 at 20 mg/kg QD for 83 days in AAV-HBV mouse serum samples. Values represent mean ± SEM of 3 mice.

CONCLUSIONS

- **CAM-E compounds showed the expected behavior in HepAD38 cells, i.e. aa reduction of HBcAg levels (DNA containing particles) and an increase of P-HBcAg levels (empty particles).**
- **CAM-A compounds showed a different profile for empty particles: the P-HBcAg levels are reduced at high concentrations but increased at lower concentrations. This could mean that the empty particles are trapped in the aggregates formed by CAM-A at a high concentration.**
- **Nucleoside analogs entecavir and TDF did not show pronounced effects on HBcAg levels and P-HBcAg levels in HepAD38 cells. However, in primary human hepatocytes entecavir did show a reduction of HBcAg and P-HBcAg levels. Similar to what we have seen in an earlier study, nucleoside analogs, TDF in this case, did induce a reduction of HBcAg and P-HBcAg in patients [2].**
- **In AAV-HBV mice, CAMs reduced both HBcAg and P-HBcAg levels measured in serum samples. The reduction was more pronounced after CAM-A treatment.**

Financial disclosure: All authors with affiliations [1] or [3] are /were directly or indirectly employed by Aligos Therapeutics Inc and may own stocks. All authors with affiliations [2] are / were directly or indirectly employed by Abbott Laboratories and may own stocks.

REFERENCES

[1] Niklasch et al 2021 Biomedicines, 9:1577.
[2] Geissler et al 2023 J Clin Virol, 166:105529.
[3] Yang et al 2014 Cell Mol Immunol, 11:71-8.
[4] Lenz et al 2019; WO2019175657A1.
[5] Debing et al 2021 EASL Congress; poster 1386.
[6] Vanrusselt et al 2024 EASL Congress; poster TOP-358-YI.
[7] Kum et al 2023 Hepatology, 78:1252-65.