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

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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.

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.

ALG-001075 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 [3] and 16 nM for ALG-001075 [4] for intracellular HBcAg. P-HBcAg levels, i.e. empty particles, increased correspondingly in medium and intracellularly. This was seen for both compound B and ALG-001075, both CAM-E compounds.

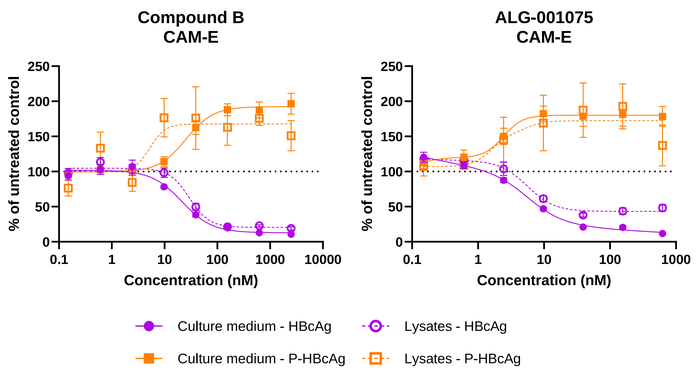

Figure 1: Dose-response curves for the effect of compound B and ALG-001075 on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from HepAD38 cells after 7 days of treatment. Values represent mean ± SEM of 2 individual experiments.

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 [5] and 260 nM for RG7907 [6] 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].

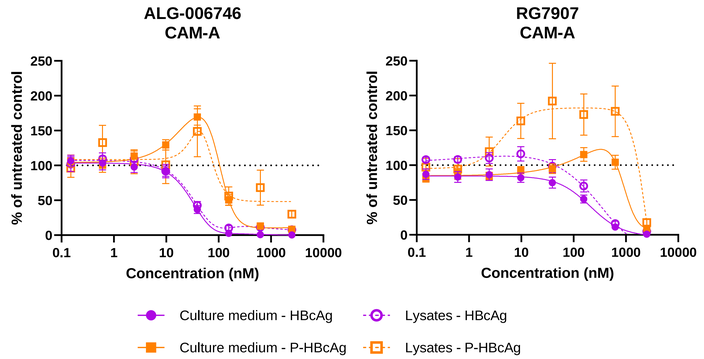

Figure 2: Dose-response curves for the effect of ALG-006746 and RG7907 on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from HepAD38 cells after 7 days of treatment. Values represent mean ± SEM of 3 individual experiments.

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.

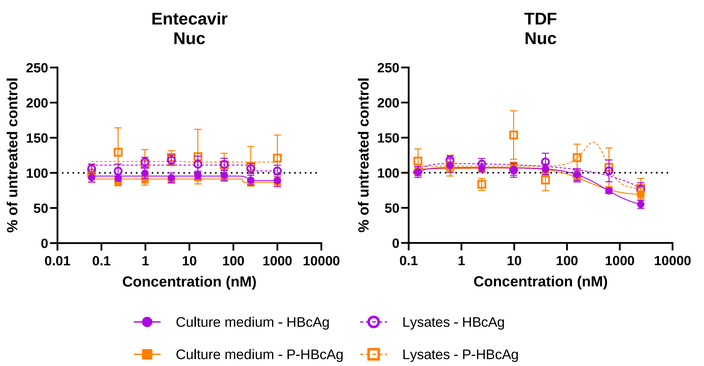

Figure 3: Dose-response curves for the effect of entecavir and TDF on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from HepAD38 cells after 7 days of treatment. Values represent mean ± SEM of 3 individual experiments.

CAMs SHOW A SLIGHTLY DIFFERENT EFFECT IN PHH

For all CAMs tested in the PHH assay, 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.

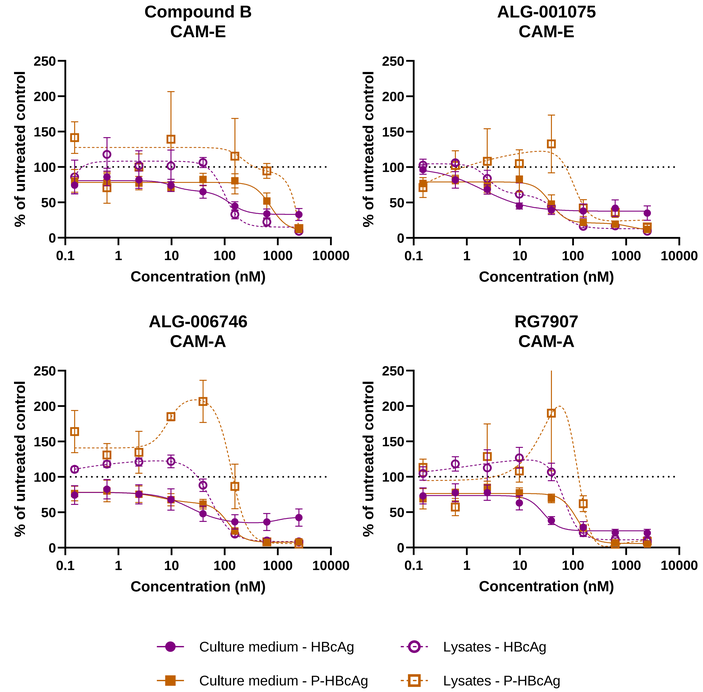

Figure 4: Dose-response curves for the effect of compound B, ALG-001075, ALG-006746 and RG7907 on HBcAg and P-HBcAg in culture medium and lysates. Results were obtained from PHH after 12 days of treatment. Values represent mean ± SEM of 2 individual experiments.

CONCLUSION

- **CAM-E compounds showed the expected behavior in HepAD38 cells, i.e. a 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. This contrasts with findings from a previous study, where treatment with the nucleotide analog TDF resulted in a reduction of HBcAg and P-HBcAg levels in patients [2].**

REFERENCES

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