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DIFFERENTIATION OF HBV CAPSID ASSEMBLY MODULATORS BASED ON STABILIZATION OF CORE PROTEIN OLIGOMERIZATION AND RESIDENCE TIME

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ALIGOS THERAPEUTICS

BACKGROUND

Despite the availability of vaccines against hepatitis B virus (HBV), no curative therapies are available for the estimated 300 million people worldwide who are chronically infected. Capsid assembly modulators (CAMs) are a promising new class of potential HBV antivirals.[1] Pevifoscorvir sodium (pevy, ALG-000184) is a prodrug of ALG-001075, a novel class E capsid assembly modulator (CAM-E). Pevy has demonstrated best-in-class reductions of HBV DNA, RNA, HBsAg, HBeAg and HBcrAg in CHB patients.[2] However, the lack of quantitative and predictive biochemical assays limits the characterization of novel and potent CAM compounds. Therefore, a novel jump dilution assay was designed to assess the capsid disassembly and the residence time of CAMs.

METHODS

Cellular antiviral activity was assessed in HepG2.117 and HepAD38 cells using intracellular HBV DNA as a readout. Capsid assembly florescence quenching assay was used to measure and classify capsid assembly. Capsid disassembly and residence time of compounds were assessed in jump dilution assays. Pharmacokinetics of compounds were studied in AAV-HBV-transduced C57Bl/6 mice.

APPARENT DISCREPANCY BETWEEN CELLULAR AND BIOCHEMICAL POTENCY OF CAMS

The cellular antiviral activity of a representative set of seven CAMs in HepG2.117 cells was determined by DNA quantification (qPCR).[3] EC_{50} values ranged from <1 nM for the most potent compound to ~ 100 nM for the least potent ones (Figure 1). Biochemical potency of CAMs was measured in the standard capsid assembly fluorescence quenching assay with BODIPY-labeled Cp150 (Cp150Bo) protein after 90 minutes of incubation[4]. Biochemical EC_{50} values ranged from 0.3-1.5 µM for all compounds, indicating that the broad range in cellular antiviral activity and high potency of some of the compounds was not reflected biochemically by capsid assembly florescence quenching (Figure 1). The apparent discrepancy and lack of correlation between biochemical and cellular data indicates that the broadly used standard capsid assembly fluorescence quenching assay is unsuitable in its current condition for potency determination.

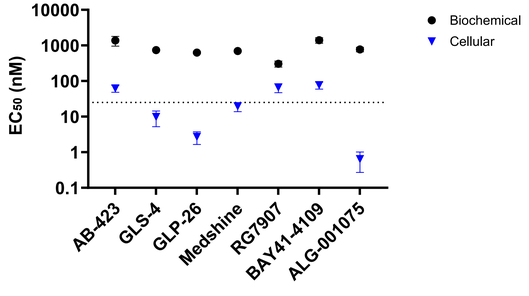


Figure 1: Comparison between cellular and biochemical potency of CAMs

EFFECT OF CAMS ON CAPSID STABILITY UNDER EQUILIBRIUM CONDITIONS

Given the shortcoming of standard capsid assembly fluorescence quenching assay. We set out to explore the possibility of developing a quantitative biochemical assay. First, we measured the effect of CAMs on capsid stability. We modified the standard fluorescence quenching assay by adding urea as a capsid destabilizing agent. In the presence of urea, the AB-423 induced capsid was completely destabilized and disassembled (Figure 2A.), in contrast, more potent molecules such as ALG-001075 have a stronger stabilizing effect, which was similar to that in the absence of urea (Figure 2B). Although the modified method could differentiate weaker compounds, the resolution was not sufficient to rank the more potent one (Figure 2C).

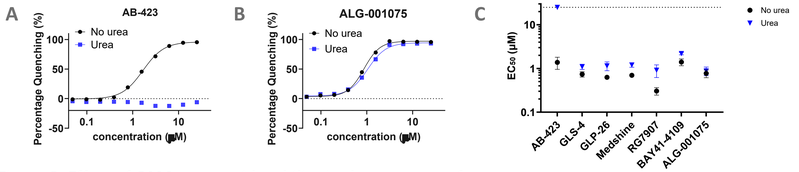
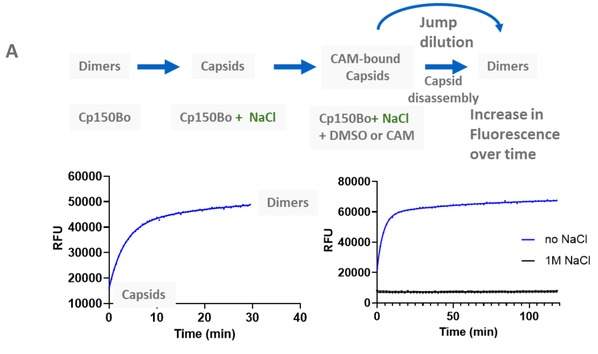


Figure 2: Effect of CAMs on capsid stability in the presence of urea

MEASUREMENT OF TIME-DEPENDENT CAPSID STABILITY (RESIDENCE TIME OF CAMS)

Inspired by jump dilution assays widely used in measuring residence time of inhibitors on enzymes[5], we further modified the standard fluorescence quenching assay to measure the effect of CAMs on the kinetics of capsid disassembly. Cp150Bo dimers (high fluorescence) were converted into capsids (low fluorescence) in the presence of 1M NaCl, after which either DMSO or CAMs were added for 30 minutes (Figure 3A). The capsids then were rapidly diluted into a hypotonic solution prior to measuring the kinetics of disassembly back into dimers, as reflected by a time-dependent gain in fluorescence. . In the absence of CAMs, the kinetics of capsid disassembly after jump dilution under hypotonic conditions was very rapid. As expected, capsid disassembly could be prevented with 1M NaCl in the dilution buffer. In the presence of CAMs, the kinetics of capsid disassembly were not significantly altered in the presence of AB-423 compared with DMSO indicating very short residence time of AB-423 on capsids, while more potent compounds showed slower dissociation and longer residence time (Figure 3B). For compounds with longest residence times, including ALG-001075, less than 50% of capsids had reverted to dimers 4 days after dilution (Figure 3C). The results suggest measuring residence time of CAMs using the jump dilution assay may provide a more quantitative method for biochemical potency determination than conventional assays.



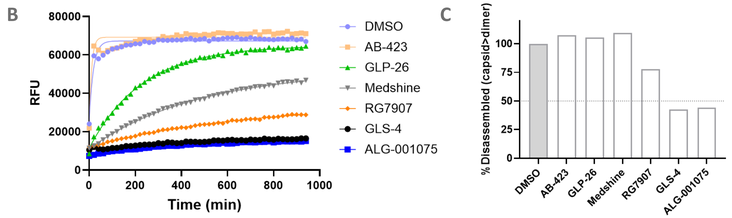


Figure 3: Development of a jump dilution assay to measure capsid disassembly over time

CORRELATION BETWEEN RESIDENCE TIME AND CELL-BASED ANTIVIRAL POTENCY

By analyzing the antiviral potency (HBV DNA) in HepG2.117 cells and residence time of CAM compounds, a previously unreported correlation between Biochemical potency and cellular antiviral was established for class E CAMs (Figure 4). The correlation highlights that ALG-001075 was among the compounds with best biochemical and cellular potencies. However, such correlation could not be established for Class A CAMs, indicating an important difference between the two compound classes (data not shown).

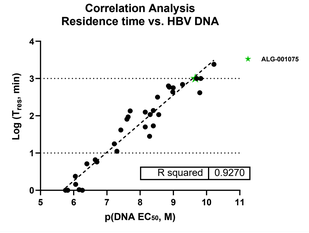


Figure 4: Correlation between cellular antiviral potency (HBV DNA) and residence time for CAM-Es.

LONG RESIDENCE TIME ALLOWS TO ACHIEVE HIGH LIVER EXPOSURE IN AAV-HBV MICE

In AAV-HBV mice expressing HBV Cp to high levels in the liver, ALG-001075 with long residence time demonstrated excellent liver exposures, when comparing to compound A (CAM-E) with a much shorter residence time (202 min).

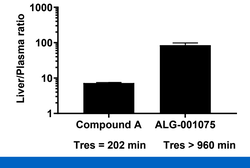


Figure 5: Liver plasma ratios of Compound A at 12 hour after last dose of 50 mg/kg BID 56-day dose duration, and ALG-001075 at 24 hour after last dose of 50 mg/kg QD 57-day dose duration.

CONCLUSION

We developed a novel jump dilution assay to assess the biochemical potency/residence time of CAM compounds. A clear correlation was established between residence time and antiviral potency of class E CAMs. The residence time of CAMs could also potentially contribute to their liver exposure. Our results demonstrate that the stability of Cp oligomerization and target residence time are important parameters for the characterization of next-generation CAMs.

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