Poster Abstract P3

Investigation of the anti-HBV activity of TL020 and its effects on host protein expression

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Background
Current drug treatments for hepatitis B virus are not sufficient to achieve desired endpoints and it is expected that a combination therapy will be important in improving patient outcomes in years to come. As such antivirals with novel mechanisms of action are promising drug leads. Cyclotriazadisulfonamide (CADA) and its analogues have shown activity against a broad range of viruses. In an *in-vitro* screen a CADA analogue, TL020, was shown to potently inhibit HBV replication with minimal effects on cellular viability. CADA has demonstrated its ability to reduce levels of specific proteins by interfering with their co-translational translocation in a signal peptide dependent manner. This interference results in mis-localization and degradation of the protein. TL020 and its effect on protein synthesis were investigated to elucidate the mechanism of its anti-HBV activity.

Methods
HepG2.2.15 cells expressing HBV virions were treated with varying concentrations of TL020 for 4 days followed by Southern blot to quantify viral DNA levels. Neutral red uptake was used to quantify toxicity. To determine the effects on cellular protein levels HepG2 cells were treated with 20 μM of TL020 for 48 hours. The cells were lysed in RIPA buffer and cellular debris separated by centrifugation. Proteins were trypsin digested and analyzed by LC-MS/MS. Alternatively after lysis, RNA was isolated using a spin column and reverse transcribed into cDNA. Transcript levels were determined by qPCR using SYBR Green dye.

Results
In the initial screen, TL020 was highly effective at reducing HBV DNA levels. The concentration required to reduce HBV DNA levels by 50% (EC_{50}) or by 90% (EC_{90}) was determined for both intracellular and excreted virions. For the excreted virions the EC_{50} was between 0.49 to 0.63 μM and the EC_{90} was between 1.2 to 1.6 μM. For intracellular HBV DNA the EC_{50} was 1.2 μM and the EC_{90} was 5.9 μM. These values were much lower than the concentration required to reduce cellular viability by 50% (CC_{50}) which was between 38 to 53 μM. Looking at the effect of TL020 on HepG2 cells using LC-MS/MS revealed significant variation in protein levels for 385 genes out of the 4,680 proteins detected. Pathway analysis revealed ECM-receptor interaction, protein processing in the ER, bile secretion, pyruvate metabolism, and metabolic pathways were the most significantly affected. In considering the mechanism for interfering with co-translational translocation, a subset of 30 proteins were down-regulated and contained signal peptides necessary for co-translation translocation. These were selected for analysis via qRT-PCR which revealed 18 proteins whose mRNA levels were either steady or increased, indicating a post-transcriptional mechanism.

Conclusions
Investigation of TL020's effect on host protein expression revealed that this analogue is likely less specific than CADA. The list of most down-regulated proteins was enriched for signal peptide-containing proteins. Follow up studies are necessary to determine which proteins are being directly targeted by the proposed mechanism, and to determine which proteins are affecting HBV replication. The work presented here is useful for prioritizing proteins for these further studies.