COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

Identification Of Verteporfin And Protoporphyrin Ix Via Medium Throughput, In Vitro Screen Where They Act As Robust, Broad-spectrum Inhibitors Of Emerging Arthropod Viruses.

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The urgency for development of effective, host-directed antivirals is paramount for both the warfighter and civilians alike. Viruses to pay careful attention to are alphaviruses as they emerge and re-emerge. Some of these viruses within this family pose an additional risk to the warfighter as they can be aerosolized and achieve rapid high titers in infected people. There are currently no FDA approved therapeutics or vaccines for alphaviral infections, and all that can be done for infected patients is offer supportive care. Additionally, the degree of symptom similarity to other arthropod-spread diseases makes discerning environmental presence and case numbers difficult. Utilizing alphaviruses, such Venezuelan equine encephalitis virus (VEEV) and Eastern equine encephalitis virus (EEEV), as surrogates for arthropod derived disease, we aim to identify small molecules of interest to repurpose and potentially employ as antivirals. Through a medium-throughput screening technique, we were able to identify several lead candidates of interest for these purposes.

Our research began with a protein-protein interacting, small molecule library consisting of 409 compounds, which were screened in vitro via a luciferase tagged VEEV TC-83. Concentration of luciferase was quantified intracellularly and used to isolate lead candidates, where viral titer was further confirmed extracellularly via plaque assay and independent assessment. Verteporfin (VP) was identified as a robust, non-toxic candidate for alphavirus inhibition, where viral load frequently fell below the limit of detection. Cytotoxicity was assessed using CellTiter-Glo assay. A literature search revealed Protoporphyrin IX (PPIX) as of interest, which showed reduced toxicity in some cell lines compared with VP and was potent inhibition of VEEV TC-83 as well. VP and PPIX have been tested in central nervous system relevant (CNS) cell lines, including astrocytes, endothelial cells, and pericytes, and act independently of cell type. Both exhibit high selectivity indices through IC50 and CC50 comparison. Broad-spectrum efficacy has since been analyzed against both Old World and New World alphaviruses, EEEV, Chikungunya (CHIKV), and the fully virulent VEEV under BSL-3 conditions.

Further work aims to identify host directed mechanisms of these small molecules at both gene and protein expression levels. VP is a powerful suppressor of YAP activity via sequestration in the cytoplasm and preventing TEAD activation. Analysis of YAP protein concentration in the context of alphavirus infection has yet to be elucidated.
Additionally, we plan to broaden our research into our gravity-fed, organ-on-a-chip model of the blood brain barrier to analyze barrier integrity in the context of infection. Furthermore, our lab has also begun work on investigating the effects of VP and PPIX on the bunyavirus, Rift Valley Fever virus and plan to further diverge into flaviviruses. We anticipate that VP and PPIX offer robust protection of cells from alphavirus infection via host modulating mechanisms. This will further elucidate the progression viral pathogenesis and how interventions can be utilized to combat infection.

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