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Virus Replication Is Inhibited By Novel Cell Penetrating Antibodies SBT-100 and SBT-106: Potential Bioterrorism Countermeasures

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BACKGROUND

Signal transducer and activator of transcription 3 (STAT3) has been shown in the literature to play an important role in the pathogenesis of multiple human and animal viruses. In addition, Ebola virus protein VP24 is an important component of the virus in its suppression of the host immune system. Singh Biotechnology's assets, SBT-100 and SBT-106 are both novel cell penetrating single domain antibodies that bind their targets with nanomolar affinity. SBT-100 binds and inhibits STAT3, and SBT-106 binds Ebola virus VP24. Using *in vitro* models, we found that SBT-100 was able to significantly suppress both Ebola virus and Zika virus replication in 48 hours. SBT-106 was specific for inhibiting Ebola virus replication in 48 hours. Because SBT-100 has been shown to cross the blood brain barrier (BBB) in a cancer tumor model, we examined its effect against the Venezuelan Equine Encephalitis virus (VEEV). Here too we saw significant suppression of VEEV replication in 48 hours.

MATERIALS & METHODS

The proprietary single domain antibodies (SBT-100 & SBT-106) to be tested were provided by Singh Biotechnology. All viruses (Ebola, Zika, VEEV, etc.) and their different strains, as well as cell lines (Vero, HeLa, HHF-1, etc.) used for viral infection done at U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). The high content cell-based screen assay protocol for testing compounds against virus infected cells was designed and developed at USAMRIID using the PerkinElmer (PE) Opera confocal assay platform. The PE Acapella software was used to compile and analyze the data generated.

RESULTS

Using a BIAcore assay, it was determined that SBT-100 binds human STAT3 with a KD = 2.24×10^{-8} M and SBT-106 binds Ebola VP24 with a KD = 2.94×10^{-8} M. To further demonstrate that SBT-100 binds STAT3, cell lysates of human breast cancer (MDA-MB-231) that constitutively expresses activated STAT3 (P-STAT3) was used. Co-immunoprecipitation and western blot studies demonstrated that SBT-100 binds STAT3. To demonstrate SBT-100 penetrates the cell membrane, MDA-MB-231 cells were cultured with SBT-100 for 6 hours and then fixed. Secondary antibody anti-llama conjugated with green fluorescence was then used to bind the SBT-100 that was already in the cytoplasm and nucleus of the cells. This resulting fluorescence gave a green speckled pattern throughout the interior of the cell. As further support, MDA-MB-231 cells were cultured with SBT-100 or S3I-201 (a known inhibitor of STAT3 phosphorylation or activation). Western blots of these cells revealed marked reduction in the levels of total STAT3

(T-STAT3) with both SBT-100 and S3I-201. Additional immunofluorescence analysis (IFA) revealed that SBT-100 caused a redistribution of STAT3 from the nucleus to the cytoplasm and reduced the amount of activated STAT3 (P-STAT3). To demonstrate that SBT-100 crosses the BBB, tumor bearing mice were injected intraperitoneally (IP) with 5 mg/kg of SBT-100 each and then 15 minutes later the mice were sacrificed. The brains and tumors were taken and stained for SBT-100. Microscopic examination revealed SBT-100 to be in the neurons and glial cells of the brains and in the cancer cells of the tumors.

The USAMRIID in vitro screening team used the PE Opera high content cell based assay confocal platform with the Acapella software to evaluate the effect of SBT-100 on viral replication and of SBT-106 on Ebola virus replication. In the HeLa cell model with Ebola virus, SBT-106 gave a 45% maximum inhibition with an EC_{50} = 3.49 µM. SBT-100 in the HeLa cell and HFF cell models with Ebola virus gave maximum inhibitions of 97% (EC_{50} = 1.27 µM) and 95% (EC_{50} = 2.56 µM) respectively. With Zika virus in the Vero cell model SBT-100 gave a maximum inhibition of 96% (EC_{50} = 0.74 µM). The VEEV in three models (HeLa, BE2M17, U87MG) with SBT-100 gave maximum inhibitions of 96% (EC_{50} = 0.665 µM), 97% (EC_{50} = 5.94 µM), and 96% (EC_{50} = 2.53 µM) respectively. Finally, Chikungunya virus in the U205 model gave a maximum inhibition of 99% (EC_{50} = 5.05 µM). The culture time for these viral assays were 48 hours.

CONCLUSION

1. SBT-100 may represent a therapeutic that can inhibit the replication of Ebola virus, Zika Virus, VEEV, and Chikungunya virus.
2. SBT-106 may represent another therapeutic that can inhibit the replication of Ebola virus.
3. Combination therapy of SBT-100 and SBT-106 may represent two different pathways for therapeutic inhibition of the Ebola Virus.
4. Because SBT-100 crosses the BBB and inhibits VEEV replication, it may represent a novel way to inhibit difficult to treat encephalitis viruses.
5. By targeting STAT3, SBT-100 may be a therapeutic agent for inhibiting the replication of those viruses that utilize STAT3 in their pathogenesis.

Learning Objectives:

Learning the use of cell penetrating antibodies for inhibiting viral replication.

Learn the effect of STAT3 inhibition in suppressing viral replication.

Learn that cell penetrating antibodies targeting VP-24 and also inhibit Ebola virus replication.