Submitted to Military Health System Research Symposium 2021

SBT-500 and SBT-501 as prophylactic drugs that prevent infection by SARS-CoV-2 and its variants

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BACKGROUND

SARS-CoV-2 and SARS-CoV-1 both infect cells via binding their Spike proteins to the hosts ACE-2 receptor. This binding mediates the entry of these viruses and their variants. Mutations in the Spike protein of the SARS-CoV-2 virus has increased efficiency in its transmissibility. Though vaccinations will help reduce infection rates, it is not yet completely clear how long the immunity from current vaccines will last and how effective they will be against ever emerging variants of this virus. SBT-500 and SBT-501 have been designed to block the SARS viruses and their variants from binding to the ACE-2 receptors. Using ELISA assay, it has been demonstrated that SBT-500 and SBT-501 block the binding of the Spike proteins from the SARS-CoV-2, SARS-CoV-1, and the UK and the South African variants of the SARS-CoV-2 virus to the ACE-2 receptor comparable to an anti-SARS-CoV-2 monoclonal antibody (positive control) derived from a patient. Furthermore, using a SARS-CoV-2 spike protein expressing pseudovirus assay, SBT-500 and SBT-501 blocked the infection of cells comparable to that of the positive control (PC).

MATERIALS & METHODS

ELISA Assay SARS-CoV-2 Inhibitor Assay. Various S1-His protein solutions (Acro Biosystems Cat # S1N-C52H3 (SARS-CoV2 S1), S1N-S52H5 (SARS S1), S1N-V52H3 (HCoV-NL63 S1), Sino Biological Cat # 40591-V08H10 (SARS-CoV-2-S1-South Africa variant B.1.351 (K417N, E484K, N501Y, D614G)) and 40591-V08H12 (SARS-CoV-2-S1-U.K. variant B.1.1.7 (ΔHV69-70, ΔY144, N501Y, A570D, D614G, P681H)) (50 µl of 6 µg/mL in TBS) were each used to coat all the wells of 3 Nunc strips (Thermo Scientific Cat # 469949) except one, which was left uncoated for a negative control. The strips were covered and incubated O/N (or 16 hours) at 4°C. The wells were washed twice with TBS and then blocked with TBS containing 0.5% (w/v) casein at room temperature for 1 hour with shaking. Biotinylated Human ACE2 (Acro Biosystems Cat# AC2-H82E6) (0.12 µg/mL in blocking solution) and the test compounds, SBT500 and SBT501, at 2 concentrations, were mixed 1:1 and incubated at room temperature for 10 minutes. The mixes (50 µl) were then added to the appropriate wells, after decanting the blocking solution. The strips were incubated at room temperature for 1 hour, with shaking. The wells were washed 4 times with TBST (TBS with 0.05% (v/v) Tween-20 (pH7.4), twice with TBS (pH 7.4) and then incubated with 50 µl Streptavidin-HRP (Acro Biosystems Cat# STN-NH115) (0.1 µg/mL in blocking solution) at room temperature for 30 min with shaking. The wells were washed, as previously described, and then incubated with shaking at RT for 5 minutes with TMB Substrate (50 µL) (Thermo Scientific Cat # 34028). The reaction was stopped with the addition of 50 µL 2M HCl and the absorbance at 450 nm was determined using a microplate spectrophotometer

(Perkin Elmer Wallac 1420-012 Victor3 Multilabel Counter). Anti-SARS-CoV-2 RBD Neutralizing antibody, Human IgG1 (Acro Biosystems Cat # SAD-S35) was used as the positive control.

SARS-CoV-2 Lentiviral Assay. ACE2-HEK293 cells (BPS Bioscience Cat # 79951) were seeded at a density of 5,000 – 10,000 cells per well into the interior 60 wells of a white clear-bottomed 96-well plate (Corning Cat# 3610) and incubated at 37°C/5% CO_2 overnight. Various concentrations of the test compound(s), each in a 5 μl volume, were mixed with 5 μl SARS-CoV-2 Lentivirus (BPS Bioscience Cat# 79942) or growth medium and then added to the appropriate wells. (Growth medium replaced the test compounds in the "no compound" control wells.) The plates were incubated for 50 - 58 hours and were assayed for luminescence using the One-Step™ Luciferase Assay System (BPS Bioscience Cat # 60690-2) following the manufacturer's protocol. ACE2-HEK293 cells were maintained and assayed in DMEM (Corning Cat# MT10013CV) containing 10% Fetal Bovine Serum (ATCC Cat# 30-2020), 0.5 μg/ml Puromycin (Selleckchem Cat# S7417), and 1% Penicillin/Streptomycin (Gibco Cat # 15140122).

RESULTS

To determine whether SBT-500 and SBT-501 inhibits the binding of ACE-2 receptor to Spike proteins from SARS viruses and their mutants, ELISA assays were used. As a positive control (PC) in the ELISA assay, an anti-SARS-CoV-2 neutralizing antibody was used. Biotinylated ACE-2 receptor binding to Spike proteins from SARS-CoV-1, SARS-CoV-2, Mutant 1 [SARS-CoV-2, Spike S1 (K417N, E484K, N501Y, D614G)], and Mutant 2 [SARS-CoV-2, Spike S1 (ΔHV69-70, ΔY144, N501Y, A570D, D614G, P681H)] were tested with SBT-500, SBT-501, and PC. Here Mutant 1 is the UK variant and Mutant 2 is the South African variant. SBT-500 and SBT-501 gave approximately 85% to 95% inhibition of the ACE-2 receptor binding to the Spike proteins of SARS-CoV-1, SARS-CoV-2, Mutant 1, and Mutant 2. The PC gave 85% to 95% inhibition of the ACE-2 receptor from binding to the Spike proteins of the SARS-CoV-2 and Mutant 2, but gave no effective blocking to the SARS-CoV-1 and Mutant 1 Spike proteins.

A SARS-CoV-2 lentivirus pseudovirus expressing unmutated Spike protein was used to bind HEK 239 cells expressing ACE-2 receptor. SBT-500 and SBT-501 each gave approximately 80% or great degree of blocking pseudovirus entry. The PC gave approximately 95% blocking of pseudovirus entry.

CONCLUSION

1. SBT-500 and SBT-501 are effective in blocking the binding of the Spike proteins of SARS-CoV-1, SARS-CoV-2 and its variants to the ACE-2 receptor.
2. SBT-500 and SBT-501 block the entry of a SARS-CoV-2 pseudovirus into human cells expressing the ACE-2 receptor.
3. SBT-500 and SBT-501 may be able to substantially reduce infection rate infection in people with exposure to SARS-CoV-1 and SARS-CoV-2 and its variants.

Learning Objectives:

Biologics, like SBT-500 & SBT-501, can be used to block the interaction of SARS-CoV-2 spike protein to the ACE2 receptor as demonstrated by the Elisa assay.

How SBT-500 & SBT-501 blocks the ability of pseudovirus expressing spike proteins to enter HEK cell expressing the ACE2 receptors.

SBT-500 & SBT-501 demonstrate that they can block the binding of the SARS-CoV-2 spike protein and its variants from binding the ACE2 receptor.