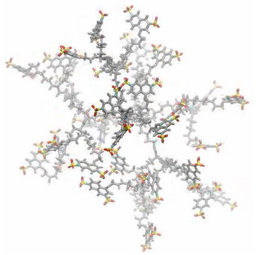

Effect of Astodrimer Sodium Against SARS-CoV-2 Variants (α, β, δ, γ, κ) In Vitro

Philippe A Gallay[1], Carolyn A Luscombe[2], Winston Stauffer[1], Michael D Bobardt[1], Graham P Heery[2], Alex Castellarnau[2], Aynaz Seta[2], Jeremy RA Paull[2].
[1]The Scripps Research Institute, La Jolla, CA, United States, [2]Starpharma Pty Ltd, Abbotsford, VIC, Australia

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BACKGROUND

The emergence of SARS-CoV-2 Variants of Concern (VOC) and Interest (VOI) have challenged the efficacy of public health strategies to control the current pandemic. Astodrimer sodium is a broad-spectrum antiviral dendrimer molecule that has been formulated as a topical nasal spray to help reduce exposure to infectious viral load in the nasal cavity. Astodrimer sodium has antiviral and virucidal activity against early pandemic isolates of SARS-CoV-2 infection and replication *in vitro*[1] and after nasal administration *in vivo*[2]. The current studies assessed the spectrum of antiviral and virucidal activity of astodrimer sodium against SARS-CoV-2 VOC, VOI and other pandemic coronaviruses.

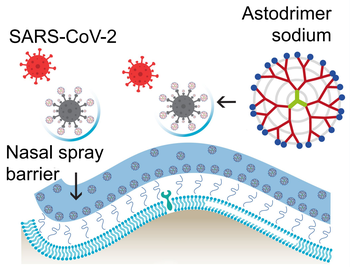
METHODS

Cell culture: Vero E6, Calu-3, or hACE2⁺ and hTMPRSS2⁺ HEK-293T cells, cultured in DMEM supplemented with 10% (v/v) heat-inactivated FBS and 1% (w/v) L-glutamine. HBBS with 2% FBS was used for infection. **Compounds:** Astodrimer sodium (SPL7013); Heparin (Sigma). **Time of addition studies:** MOI 0.01 PFU/cell or 1 ng SARS-CoV-2 nucleocapsid/cell. **Virucidal studies:** 10 mg/mL astodrimer sodium incubated with 10^5 SARS-CoV-2 PFU for 0.5, 1, 5, 15 and 30 min then neutralized as previously described[1]. Cell culture supernatant harvested 16 h post-infection, infectious progeny virus quantitated by plaque assay in Vero E6 cells[1]. **SARS-CoV-2:** USA/WA-1/2020 (NR-52281); α: hCoV-19/England/204820464/2020 (NR-54000); β: hCoV-19/South Africa/KRISP-K005325/2020 (NR-54009); δ: hCoV-19/USA/PHC658/2021 (NR-55611); γ: hCoV-19/Japan/TY7-503/2021 (NR-54982); κ: hCoV-19/USA/CA-Stanford-15_S02/2021 (NR-55486) (BEI Resources). **Pseudoviruses (confocal microscopy, FACS analysis):** Pseudotyped SARS-CoV-1 (Urbani), SARS-CoV-2 (Wuhan-Hu-1), and MERS-CoV (HCoV-EMC) lentivirus RVPs generated by Integral Molecular (Philadelphia, PA, USA) (RVP-801, RVP-701, RVP-901, respectively). Replication incompetent SARS-CoV-2 RVPs display antigenically correct spike protein including receptor binding domain (RBD). **Viral spike RBD or S1 (binding studies):** SARS-CoV-2 spike S1/RBD of USA/WA-1/2020, SARS-CoV-2 Wuhan-Hu-1, and spike S1 or RBD from VOC-α, β and γ. USA/WA-1/2020 spike S1-His tagged donated by Serhat Gumrukcu (Seraph Research Institute CA, USA). SARS-CoV-2 His-tagged α spike S1 (40591-V08H10), β spike S1 (40591-V08H10) and γ spike RBD (40592-V08H86) (SinoBiological).

References:
1. Paull JRA, Heery GP, Bobardt MD, Castellarnau A, Luscombe CA, Fairley JK, Gallay PA. 2021. Virucidal and antiviral activity of astodrimer sodium against SARS-CoV-2 in vitro. *Antiviral Res.* **191**:105089.
2. Paull JRA, Luscombe CA, Castellarnau A, Heery GP, Bobardt M, Gallay P. 2021. Protective effects of astodrimer sodium 1% nasal spray formulation against SARS-CoV-2 nasal challenge in K18-hACE2 mice. *Viruses* **13**:1656.

Astodrimer sodium is a potent antiviral and virucidal barrier to SARS-CoV-2 early pandemic isolates, VOC α, β, δ, γ, and VOI κ replication *in vitro*.

Astodrimer sodium acts as a barrier between the viral 'spike' proteins and the cell membrane, trapping and irreversibly inactivating viral particles – blocking virus from attaching to and entering the human cells



RESULTS

Time of Addition Studies

Astodrimer sodium substantially reduced viral replication of SARS-CoV-2 early pandemic isolate, and all VOC/VOI in Vero E6 (Fig 1) and Calu-3 cells. Astodrimer sodium was most effective against more infectious VOC (α, δ), and when added 1 h prior to or at the time of infection versus 1 h post-infection.

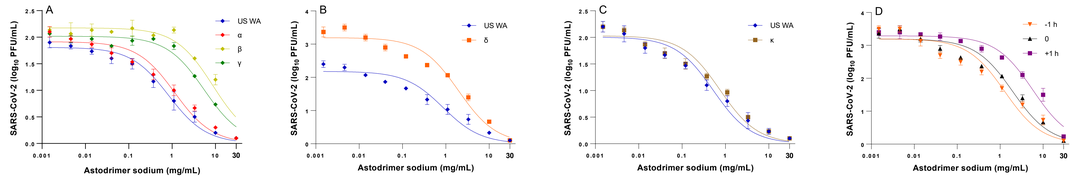


Figure 1. A, B and C: Dose-response antiviral evaluation of astodrimer sodium against SARS-CoV-2 USA-WA-1/2020 and VOC (α, β, δ, and γ) and VOI (κ). Astodrimer sodium added 1 h prior to infection. Figure 1. D: Dose-response antiviral evaluation of astodrimer sodium added 1 h prior to, at time of, or 1 h post-infection against SARS-CoV-2 VOC δ. Antiviral efficacy measured by reduced progeny infectious virus (log_{10} PFU/mL) in Vero E6 cells, Day 2 post-infection. Mean ± SD.

Virucidal Evaluation

Astodrimer sodium demonstrated potent virucidal activity against SARS-CoV-2 VOC α, β, δ and γ, and VOI κ in Vero E6 (Table 1) and Calu-3 cells with as little as 30 sec incubation time. Astodrimer sodium reduced infectious viral load of all variants by >99.99% (>4 logs) vs virus control.

Table 1. Virucidal evaluation of astodrimer sodium (10 mg/mL) incubated with SARS-CoV-2 prior to neutralization and virus exposure to Vero E6 cells. Astodrimer sodium virucidal efficacy measured by percent reduction of progeny infectious SARS-CoV-2 versus untreated virus control.

Virus:Astodrimer Sodium (10 mg/mL) Incubation Time	Percent Reduction of Infectious Progeny Virus vs Untreated Virus Control					
	USA/WA-1/2020	Alpha	Beta	Gamma	Delta	Kappa
30 seconds	>99.9%	>99.9%	>99%	>99%	>99.99%	>99.9%
1 minute	>99.9%	>99.9%	>99%	>99%	>99.99%	>99.9%
5 minutes	>99.9%	>99.99%	>99.9%	>99.9%	>99.99%	>99.9%
15 minutes	>99.99%	>99.99%	>99.99%	>99.99%	>99.999%	>99.99%
30 minutes	>99.99%	>99.99%	>99.99%	>99.99%	>99.999%	>99.99%

RESULTS

SARS-CoV-2 spike/RBD binding

Astodrimer sodium inhibited the binding of SARS-CoV-2 early pandemic strain S1 to hACE2 (Fig 2) but did not prevent binding to the SARS-CoV-2 α (S1), β (S1) and γ (RBD). Astodrimer sodium potentially interacts at up to 4 sites on each spike protein, with one site being in the RBD/S1.

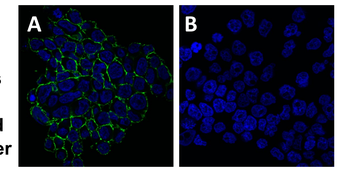


Figure 2. Confocal microscopy of SARS-CoV-2 US-WA1/2020 (S1) proteins binding to HEK-293T cells expressing hACE2⁺ receptor and hTMPRSS2⁺ in the absence (A) and presence (B) of 1 mg/mL astodrimer sodium. Magnification x63.

Pan-Coronavirus Spike/RBD binding studies

Astodrimer sodium inhibits the binding of the spike proteins of SARS-CoV-1 and -2 as well as MERS-CoV in Vero E6 cells (Fig 3).

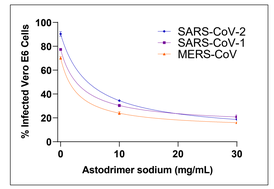


Figure 3. Dose-response inhibition by astodrimer sodium of SARS-CoV-2 (Wuhan-Hu-1), SARS-CoV-1 (Urbani), MERS-CoV (HCoV-EMC) spike proteins binding to their receptors on Vero E6 cells. Percent of cells infected with pseudotyped viruses measured by FACS.

Astodrimer sodium is more effective at blocking SARS-CoV-2 (Wuhan-Hu-1) spike RBD to the ACE2 receptor than heparin (Fig 4).

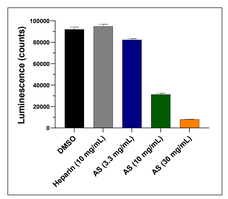


Figure 4. Astodrimer sodium (AS) inhibition of ACE-2 receptor binding to SARS-CoV-2 (Wuhan-Hu-1) spike RBD by ELISA compared heparin and DMSO negative control.

CONCLUSIONS

Astodrimer sodium mimics negatively charged heparan sulfate, which is used by viruses to concentrate at a cell surface, and provides a potent antiviral and virucidal barrier to viral attachment and entry into a host cell.

The potent, broad-spectrum, anti-pandemic coronavirus and virucidal efficacy of astodrimer sodium against whole virus is likely due to blocking multiple electrostatic interactions of the spike protein that are not negated by minor or major changes to the isolated RBD of SARS-CoV-2 VOC α, β and γ alone. Mutations that make variants more infectious appear to increase the ability of astodrimer sodium to block virus. Astodrimer sodium has the potential to block pan-SARS-CoV-2, thus reducing potential for COVID-19.

ADDITIONAL KEY INFORMATION

Corresponding author: jeremy.paull@starpharma.com

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