Short-form paper

Niclosamide shows strong antiviral activity in a human airway model of SARS-CoV-2 infection and a conserved potency against the UK B.1.1.7 and SA B.1.351 variant

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Running title: Niclosamide is active against SARS-CoV-2 variants

Abstract

SARS-CoV-2 variants are emerging with potential increased transmissibility highlighting the great unmet medical need for new therapies. Niclosamide is a potent anti-SARS-CoV-2 agent that has advanced in clinical development. We validate the potent antiviral efficacy of niclosamide in a SARS-CoV-2 human airway model. Furthermore, niclosamide is effective against the D614G, B.1.1.7 and B.1.351 variants. Our data further support the potent anti-SARS-CoV-2 properties of niclosamide and highlights its great potential as a therapeutic agent for COVID-19.

Keywords: COVID-19, small molecule, niclosamide, HAE model, variants of concern, SARS-CoV-2

Main Body

Since its emerge in 2019, coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to over 3.1 million deaths worldwide as of April 26, 2021 (1). A tremendous joint research effort led to the approval of several vaccines at unprecedented speed yet anti-viral treatment options remain limited. At the same time, several viral variants harboring mutations in the N-terminal (NTD) and receptor-binding domain (RBD) of the spike protein gene, such as the B.1.1.7 (also named 20I/501Y.V1), B.1.351 (also named 20H/501Y.V2) variants, are causing global concern as they have been associated with enhanced transmissibility and possible resistance to vaccines and antibody neutralization (2–6). The B.1.1.7 and B.1.351 lineages have been linked to a ~50% increased transmission of SARS-CoV-2 infection and the vaccine efficacy of ChAdOx1 nCoV-19 has been reported to be reduced to 10.4% against the B.1.351 variant (6–9). Thus, despite the recent vaccine roll-out, there remains a high unmet need for novel therapeutics against SARS-CoV-2, which should be effective against circulating and potentially emerging variants of concern of SARS-CoV-2.

Niclosamide has been identified as a potent inhibitor of SARS-CoV-2 *in vitro* and *in vivo* and its optimized formulation for intranasal application and inhalation, was well-tolerated in healthy volunteers in a Phase 1 trial (10–13). Herein, we sought to further characterize the anti-viral properties of niclosamide by determining its potency in a human epithelial airway model of SARS-CoV-2 infection and tested its efficacy against several variants of concern of SARS-CoV-2.

To strengthen the existing data on the potent antiviral activity of niclosamide with a preclinical model resembling the human respiratory tract, we employed a trans-well bronchial human airway epithelium (HAE) model infected with SARS-CoV-2. HAE cultured at an airway-liquid interface has been extensively used as an *in vitro* physiological model mimicking the human mucociliary airway epithelium to validate the effectivity of antivirals on infections in conducting airways (14–16). The effect of niclosamide on the

61 replication of SARS-CoV-2 in the HAE bronchial model (Epithelix) was determined as previously described
62 by Touret *et al*. (17) and Pizzorno *et al*. (14).
63 Briefly, human bronchial epithelial cells were apically infected with the European D614G strain of SARS-
64 CoV-2 (BavPat1/2020; obtained from EVA-GLOBAL) at a MOI of 0.1 and cultivated in basolateral media
65 that contained different concentrations of niclosamide (in duplicates) or no drug (virus control) for up to 4
66 days. Media was renewed daily containing fresh niclosamide. Remdesivir was used as experimental positive
67 control and non-treated samples as negative control. On day 4, samples were collected at the apical side and
68 the viral titer was estimated with a $TCID_{50}$ assay. Then, cells were lysed, and the intracellular viral RNA
69 was extracted and quantified by qRT-PCR. The viral inhibition was calculated with the infectious titers by
70 normalizing the response, having the bottom value as 100% and top value as 0%. The IC_{50} was determined
71 using logarithmic interpolation (Y=100/(1+10^((LogEC50-X)*HillSlope) in GraphPad Prims 7. Statistical
72 analysis was performed using the Ordinary One-way Anova with Dunnett's multiple comparisons test.
73
74 Niclosamide exhibited anti-SARS-CoV-2 activity by reducing the infectious titer and intracellular RNA
75 levels in the HAE model in a dose-responsive manner. Niclosamide treatment with concentrations ≥ 1 µM
76 significantly reduced the infectious titer to below the level of detection at Day 4 post-infection, yielding an
77 IC_{50} of 0.96 µM (Fig. 1A and 1C). Furthermore, treatment with concentrations ≥ 1 µM of niclosamide
78 significantly reduced the intracellular viral RNA level reaching a maximum effect of a 3-fold reduction on
79 Day 4 (Fig. 1B). These data validate the substantial anti-SARS-CoV-2 effect of niclosamide in a
80 reconstituted human airway model.
81
82 We then tested the activity of niclosamide against several variants of concern of SARS-CoV-2, including
83 the BavPat1 strain (D614G), SARS-CoV-2 201/501YV.1 (UVE/SARS-CoV-2/2021/FR/7b; lineage
84 B.1.1.7, ex UK), SARS-CoV-2 Wuhan D614, and SARS CoV-2 SA lineage B.1.351 (UVE/SARS-CoV-
85 2/2021/FR/1299-ex SA) in VeroE6 TMPRSS2 cells (ID 100978, CFAR). All viruses were obtained through
86 EVA GLOBAL. The IC50 were determined by RT-qPCR as previously described by Touret *et al.* (18).

87 Briefly, eight 2-fold serial dilutions of niclosamide in triplicate were added to the cells 15 min prior to viral
88 infection and incubated for 2 days at 37°C. Remdesivir was used as experimental positive control and non-
89 treated samples as negative control. The viral genome was quantified by real-time RT-qPCR from the cell
90 supernatant (17). The IC50 was calculated as described above. All data associated with this study are present
91 in the paper.
92
93 Niclosamide inhibited replication of the SARS-CoV-2 original strain (Wuhan D614) in VeroE6 TMPRSS2
94 cells with an IC_{50} of 0.13µM and IC_{90} of 0.16 µM which is in accordance with previous studies (10, 11).
95 Importantly, niclosamide also blocked the replication of the European BavPat D614G, UK B.1.1.7 and SA
96 B.1.351 variant with an IC_{50} of 0.06 µM, 0.08 µM and 0.07 µM, respectively (Fig. 2). Thus, niclosamide is
97 effective against all tested variants of SARS-CoV-2 having a similar potency across the different strains
98 compared to the original Wuhan D614 strain.
99
100 These data are in line with the host-targeted mode of action of niclosamide, which has been described to
101 interfere with basic cellular mechanisms involved in SARS-CoV-2 replication, such as autophagy, the
102 endosomal pathway and the TMEM16A chloride channel (11, 19–21). Accordingly, niclosamide is a potent
103 antiviral therapeutic agent against SARS-CoV-2 and its variants. The molecule will also deserve further
104 investigations to assess its potential role in the chemotherapeutic armamentarium required for future
105 emerging infectious disease preparedness.
106
107 Taken together, our findings support niclosamide's therapeutic potential as a potent anti-viral agent against
108 SARS-CoV-2, including its variants of concern. Trials in patients with COVID-19 are needed to substantiate
109 future clinical use.
110

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References

1. Dong E, Du H, Gardner L. 2020. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis.

2. Mahase E. 2021. Covid-19: Novavax vaccine efficacy is 86% against UK variant and 60% against South African variant. BMJ. BMJ Publishing Group.

3. Johnson & Johnson Announces Single-Shot Janssen COVID-19 Vaccine Candidate Met Primary Endpoints in Interim Analysis of its Phase 3 ENSEMBLE Trial | Janssen.

4. Wang P, Liu L, Iketani S, Luo Y, Guo Y, Wang M, Yu J, Zhang B, Kwong PD, Graham BS, Mascola JR, Chang JY, Yin MT, Sobieszczyk M, Kyratsous CA, Shapiro L, Sheng Z, Nair MS, Huang Y, Ho DD. 2021. Increased Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7 to Antibody Neutralization. bioRxiv Prepr Serv Biol 2021.01.25.428137.

5. Xie X, Zou J, Fontes-Garfias CR, Xia H, Swanson KA, Cutler M, Cooper D, Menachery VD, Weaver S, Dormitzer PR, Shi P-Y, Philip P. 2021. Neutralization of N501Y mutant SARS-CoV-2 by BNT162b2 vaccine-elicited sera. bioRxiv 2021.01.07.425740.

6. Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L, Padayachee SD, Dheda K, Barnabas SL, Bhorat QE, Briner C, Kwatra G, Ahmed K, Aley P, Bhikha S, Bhiman JN, Bhorat AE, du Plessis J, Esmail A, Groenewald M, Horne E, Hwa S-H, Jose A, Lambe T, Laubscher M, Malahleha M, Masenya M, Masilela M, McKenzie S, Molapo K, Moultrie A, Oelofse S, Patel F, Pillay S, Rhead S, Rodel H, Rossouw L, Taoushanis C, Tegally H, Thombrayil A, van Eck S, Wibmer CK, Durham NM, Kelly EJ, Villafana TL, Gilbert S, Pollard AJ, de Oliveira T, Moore PL, Sigal A, Izu A. 2021. Efficacy of the ChAdOx1 nCoV-19 Covid-19 Vaccine against the B.1.351 Variant. N Engl J Med NEJMoa2102214.

7. Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Hopkins S, Gandy A, Rambaut A, Ferguson NM. 2021. Transmission of SARS-CoV-2 Lineage B.1.1.7 in England: Insights from linking epidemiological and genetic data. medRxiv 2020.12.30.20249034.

8. Davies NG, Barnard RC, Jarvis CI, Kucharski AJ, Munday J, Pearson CAB, Russell TW, Tully DC,

Abbott S, Gimma A, Waites W, Wong KLM, van Zandvoort K, Eggo RM, Funk S, Jit M, Atkins KE, Edmunds WJ, Houben R, Meakin SR, Quilty BJ, Liu Y, Flasche S, Lei J, Sun FY, Krauer F, Lowe R, Bosse NI, Nightingale ES, Sherratt K, Abbas K, O'Reilly K, Gibbs HP, Villabona-Arenas CJ, Waterlow NR, Medley G, Brady O, Williams J, Rosello A, Klepac P, Koltai M, Sandmann FG, Foss AM, Jafari Y, Prem K, Chan YWD, Hellewell J, Procter SR, Jombart T, Knight GM, Endo A, Quaife M, Showering A, Clifford S. 2020. Estimated transmissibility and severity of novel SARS-CoV-2 Variant of Concern 202012/01 in England. medRxiv. medRxiv.

9. Pearson CA. Estimates of severity and transmissibility of novel South Africa SARS-CoV-2 variant 501Y.V2.

10. Jeon S, Ko M, Lee J, Choi I, Byun SY, Park S, Shum D, Kim S. 2020. Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs. Antimicrob Agents Chemother https://doi.org/10.1128/AAC.00819-20.

11. Nils C. Gassen, Papies J, Bajaj T, Dethloff F, Emanuel J, Weckmann K, Heinz DE, Lennarz HM, Richter A, Niemeyer D, Corman VM, Giavalisco P, Drosten C, Müller MA. 2020. Analysis of SARS-CoV-2-controlled autophagy reveals spermidine, MK-2206, and niclosamide as putative antiviral therapeutics. bioRxiv.

12. Backer V, Sjöbring U, Sonne J, Weiss A, Hostrup M, Johansen HK, Becker V, Sonne DP, Balchen T, Jellingsø M, Sommer MOA. 2021. A randomized, double-blind, placebo-controlled phase 1 trial of inhaled and intranasal niclosamide: A broad spectrum antiviral candidate for treatment of COVID-19. Lancet Reg Heal Eur 100084.

13. Brunaugh AD, Seo H, Warnken Z, Ding L, Seo SH, Smyth HDC. 2020. Broad-Spectrum, Patient-Adaptable Inhaled Niclosamide-Lysozyme Particles are Efficacious Against Coronaviruses in Lethal Murine Infection Models. bioRxiv.

14. Pizzorno A, Padey B, Julien T, Trouillet-Assant S, Traversier A, Errazuriz-Cerda E, Fouret J, Dubois J, Gaymard A, Lescure FX, Dulière V, Brun P, Constant S, Poissy J, Lina B, Yazdanpanah Y, Terrier O, Rosa-Calatrava M. 2020. Characterization and Treatment of SARS-CoV-2 in Nasal and Bronchial

Human Airway Epithelia. Cell Reports Med https://doi.org/10.1016/j.xcrm.2020.100059.

15. Boda B, Benaoudia S, Huang S, Bonfante R, Wiszniewski L, Tseligka ED, Tapparel C, Constant S. 2018. Antiviral drug screening by assessing epithelial functions and innate immune responses in human 3D airway epithelium model. Antiviral Res https://doi.org/10.1016/j.antiviral.2018.06.007.

16. Sheahan TP, Sims AC, Zhou S, Graham RL, Pruijssers AJ, Agostini ML, Leist SR, Schafer A, Dinnon KH, Stevens LJ, Chappell JD, Lu X, Hughes TM, George AS, Hill CS, Montgomery SA, Brown AJ, Bluemling GR, Natchus MG, Saindane M, Kolykhalov AA, Painter G, Harcourt J, Tamin A, Thornburg NJ, Swanstrom R, Denison MR, Baric RS. 2020. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med https://doi.org/10.1126/SCITRANSLMED.ABB5883.

17. Touret F, Driouich JS, Cochin M, Petit PR, Gilles M, Barthélémy K, Moureau G, Malvy D, Solas C, de Lamballerie X, Nougairède A. 2020. Preclinical evaluation of Imatinib does not support its use as an antiviral drug against SARS-CoV-2. bioRxiv.

18. Touret F, Gilles M, Barral K, Nougairède A, van Helden J, Decroly E, de Lamballerie X, Coutard B. 2020. In vitro screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. Sci Rep 10:13093.

19. Braga L, Ali H, Secco I, Chiavacci E, Neves G, Goldhill D, Penn R, Jimenez-Guardeño JM, Ortega-Prieto AM, Bussani R, Cannatà A, Rizzari G, Collesi C, Schneider E, Arosio D, Shah AM, Barclay WS, Malim MH, Burrone J, Giacca M. 2021. Drugs that inhibit TMEM16 proteins block SARS-CoV-2 Spike-induced syncytia. Nature 1–8.

20. Jurgeit A, McDowell R, Moese S, Meldrum E, Schwendener R, Greber UF. 2012. Niclosamide Is a Proton Carrier and Targets Acidic Endosomes with Broad Antiviral Effects. PLoS Pathog 8:e1002976.

21. Xu J, Shi PY, Li H, Zhou J. 2020. Broad Spectrum Antiviral Agent Niclosamide and Its Therapeutic Potential. ACS Infect Dis 6:909–15.

193 **Figures**

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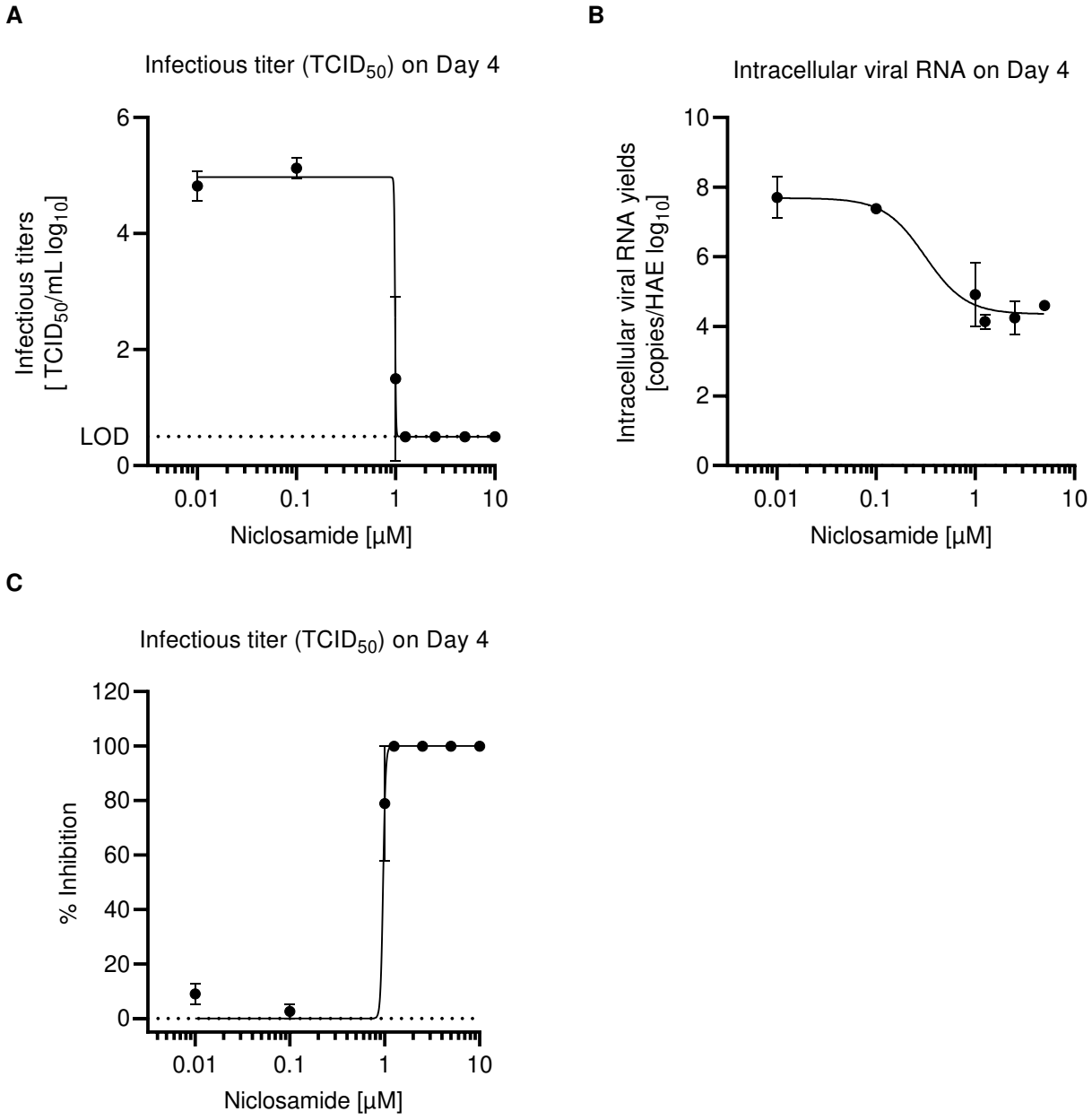
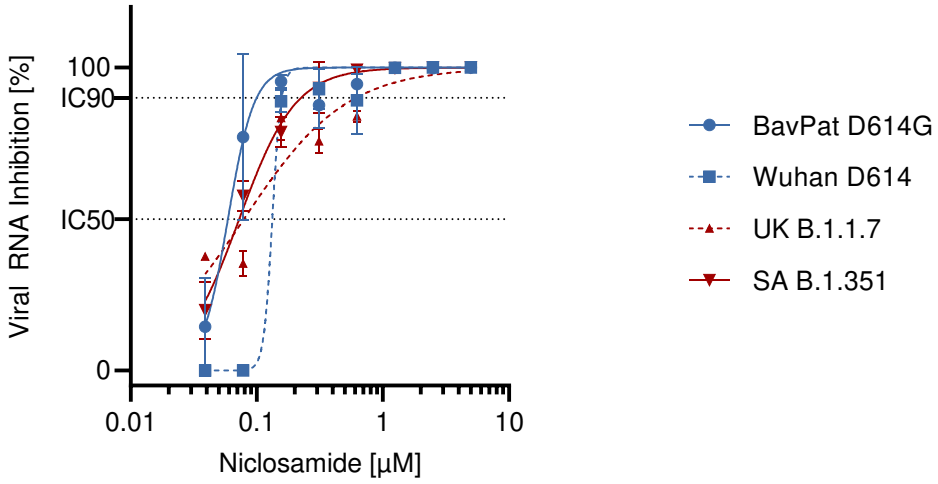


195

196 **Figure 1: Antiviral efficacy of niclosamide in a trans-well model of human bronchial epithelium**
197 **infected with SARS-CoV-2.** Dose-dependent effects of niclosamide on infectious titer of SARS-CoV-2
198 (A) and intracellular viral RNA levels (B) on Day 4 post-infection. The reduction of infectious titer and
199 intracellular RNA was significant for concentrations ≥ 1 µM niclosamide (infectious titer: 1 µM = $p < 0.05$,
200 1.25 – 10 µM = $p < 0.0001$; intracellular viral RNA: 1, 2.5, 5 µM = $p < 0.01$, 1.25 = $p < 0.001$ compared to

non-treated control; Ordinary One way Anova with Dunnett's multiple comparisons test). The IC_{50} based on the infectious titer on Day 4 was 0.96 µM (C). N = 2



	BavPat D614G	Wuhan D614	UK B.1.1.7	SA B.1.351
IC50 [µM]	0.06	0.13	0.08	0.07

203

204 **Figure 2: Effect of niclosamide on SARS-CoV-2 variants, including UK B.1.1.7 and SA B.1.351 in**

205 **VeroE6 TMPRSS2 cells.** IC = Inhibitory concentration. The origin of the tested variants is available at

206 EVA-GLOBAL. N = 3