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p38 MAPK Inhibitor SB203580 Suppresses SARS-CoV-2 Replication

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the causative agent of Coronavirus disease (COVID-19) resulted in loss of 6.95 million of human lives. Although vaccines to induce immune-prophylaxis against COVID-19 have been developed, there is no specific antiviral drug to treat COVID-19. A library of small molecule chemical inhibitors was screened comprising the inhibitors targeting cellular kinases and phosphatases, and identified SB203580 [an inhibitor of p38 mitogen activated kinase (p38 MAPK)] as one of the hits against SARS-CoV-2. The non-cytotoxic dose (8µg/ml) of SB203580 significantly reduced SARS-CoV-2 titre (≥ log2 fold) in absence of any virucidal activity. This study identified p38 MAPK inhibitor SB203580 as a potential host-directed antiviral agent against SARS-CoV-2.

Key words: SARS-CoV-2, p38 MAP Kinase, SB203580, antiviral efficacy, virucidal

INTRODUCTION

The SARS-CoV-2, the causative agent of the coronavirus 2019 (COVID-19) disease (Esai Selvan, 2020) infected 768,560,727 persons including 6,952,522 deaths till August 2023. The current vaccines are effective but the emergence of mutations in spike protein have increased virus transmission and re-infection and reduced protection from neutralizing antibodies (Parums, 2021). However, there are no effective medications available for the treatment of COVID-19. Remdesivir, which is used to treat Ebola infections also showed antiviral action against SARS-CoV and MERS-CoV-2 (Warren *et al.*, 2016). The remdesivirtargets viral RNA dependent RNA polymerase (RdRp) gene (Wang *et al.*, 2020). Besides, ivermectin, lopinavir/ritonavir, chloroquine, hydroxychloroquine, azithromycin and other viral protease inhibitors treatment with interferons or nitazoxanide also seems to act against SARS-CoV-2 but are not recommended for use in non-hospitalized or hospitalised COVID-19 patients (Lemaitre *et al.*, 2022). Moreover, these are prone to induce drug-resistant vial variants. p38-MAPK (a cellular kinase) activated during the stress and immflamatory responses (Whitaker and Cook, 2021). It has four isoforms: p38α, p38β, p38δ and p38γ controlling many cellular processes (Canovas and Nebreda, 2021). p38 is activated by its upstream MAPK: MEEK3 and MEKK6. Once p38 is activated it phosphorylates more than 100 proteins (Martínez-Limón *et al.*, 2020). The present study screened 108 small molecule chemical inhibitors known to target cellular kinases, phosphatases and epigenetic modifiers. Out of this Pyridinyl imidazole inhibitor SB203580 against p38 mitogen-activated protein (p38-MAPK) was found as a potential hit against SARS-CoV-2.

MATERIALS AND METHODS

Vero (African green monkey kidney) cells, available at National Centres for Veterinary Type Culture (NCVTC), Hisar were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with antibiotics and 10% heat-inactivated fetal bovine serum (FBS; Sigma, St. Louis, USA) and antibiotics (Penicillin and Streptomycin). Wild type (SARS-CoV-2/Human-tc/India/2020/Hisar-4907) SARS-CoV-2

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(GenBank Accession Number of MW555598) was available at NCVTC, Hisar. Virus was propagated in Vero cells in the Biosafety level 3 (BSL-3) laboratory of ICAR-National Research Centre on Equines (NRCE), Hisar, India (Khandelwal *et al.*, 2021). The virus was quantified by plaque assay and viral titres were measured as plaque forming unit per millilitre (PFU/ml).

Adezmapimod (SB203580), IUPAC name 4-(4'-Fluorophenyl)-2-(4'-methylsulfinylphenyl)-5-(4'-pyridyl)-imidazole a pyridinyl imidazole inhibitor were procured from Sigma (Steinheim, Germany) widely used in the role of p38-Mitogen activated protein kinases (MAPK). SB203580 inducing mitophagy and autophagy. DMSO was used as a vehicle control for the inhibitors (Fig. 1).

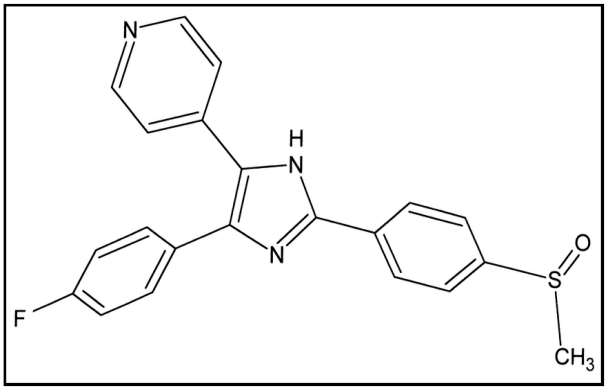

Fig. 1. Adezmapimod (SB 203580) is a selective and ATP-competitive p38 MAPK inhibitor. 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole. SB203580 a pyridinyl imidazole which specifically inhibits the activity of p38 MAPK but not its activation by upstream MAPKK.

For determination of the cytotoxicity, Vero cells in 96-well tissue culture plates, in triplicates, were treated with 5-fold serial dilution of SB203580 or vehicle control (DMSO) for 96 h and cytotoxity was determined by MTT assay (Kumar *et al.*, 2019).

Confluent monolayers of Vero cells were infected at MOI 0.1 with SARS-CoV-2, followed by washing with PBS and addition of fresh DMEM containing SB203580 (8 µg/ml) or equivalent volume of DMSO. At 24 h post infection (hpi), virus particles released in the supernatant were quantified by plaque assay. For determination of Virucidal activity, SARS-CoV-2 (~10^7 PFU each) was incubated with 5-fold serial dilutions of the inhibitor (SB203580) for 90 min. Thereafter, residual viral infectivity was determined by plaque assay (Khandelwal *et al.*, 2020).

RESULTS AND DISCUSSION

For determining the *in vitro* efficacy of SB203580 against SARS-CoV-2, the effect of SB203580 on Vero cells by MTT [(3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide)] assay was evaluated (Fig. 2). SB203580 did not significantly affect the Vero cells between 8 to 0.0128 µg/ml when incubated for 96 h. However, at higher concentrations, SB203580 had toxic effect. The cytotoxic concentration 50 (CC_{50}) was determined as 64.50±5.9 µg/ml. Further to check the antiviral efficacy of SB203580 against SARS-CoV-2, anon-cytotoxic concentrations of SB203580 (8 µg/ml), when applied to the SARS-CoV-2-infected Vero cells, resulted in ~ 100-fold reduction in SARS-CoV-2 yield (Fig. 3). To check the virucidal activity of extracellular virions, SARS-CoV-2 was incubated with various concentrations of SB203580. The infectious viral particles were compared in drug treated and dimethyl sulfoxide (DMSO)-treated cells (Fig. 4), suggesting that SB203580 did not exert virucidal activity on SARS-CoV-2. p38-MAPK was a major pathway which was involved in induction of cytokines (Chander *et al.*, 2021).

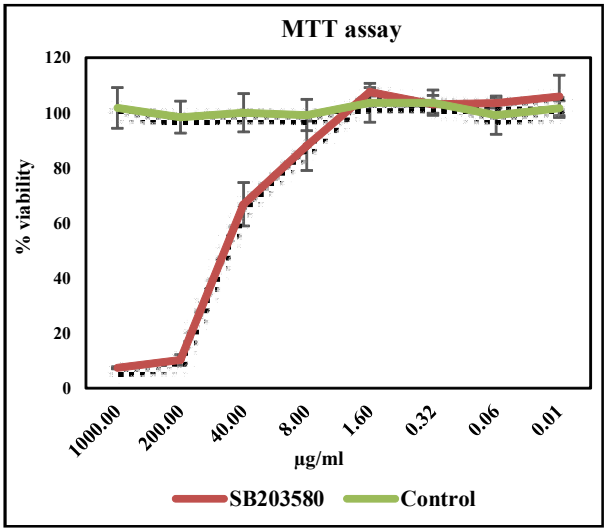

Fig. 2. Determination of the cytotoxicity (MTT assay) indicating concentrations of SB203580 or equivalent volumes of vehicle control (PBS) incubated with cultured Vero cells for 96 h and percentage of cell viability determined by MTT assay. CC_{50} determined by Reed-Muench method.

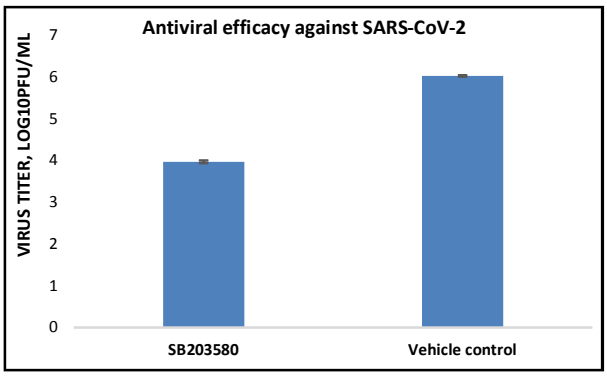

Fig. 3. *In-vitro* antiviral efficacyVero cells, in triplicates, were infected with SARS-CoV-2 at MOI of 0.1 in the presence of 8 µg/ml of SB203580 or equivalent volume of DMSO. At 24 hpi, infectious virus particles released in the infected cell culture supernatants were quantified by plaque assay.

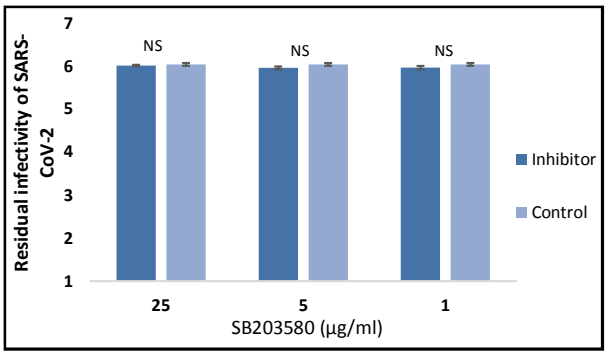

Fig. 4. Inactivation concentration 50 (IC50) indicating concentrations of the SB203580 or equivalent volumes of PBS mixed with 10^6 pfu of SARS-CoV-2 and incubated for 90 min at 37°C after which residual viral infectivity (% of control) determined by plaque assay.

Although the cytokine induction was aimed at limiting the virus replication, but over induction of the cytokine, also called as cytokine storm, leading to immune-pathology. In this regard, SB203580 (p38 MAPK inhibitor) may have dual effect, it restricts virus replication and may dampen cytokine storm (Grimes and Grimes, 2020) which is a major risk factor in COVID-19 induced death. In previous studies, p38 inhibition was shown to reduce the severity of the illness caused by dengue virus (DENV) in mice (Sreekanth *et al.*, 2018). The majority of FDA-approved antiviral medications targeted virally encoded genes (Wu *et al.*, 2020), which resulted in drug-resistant virus variations (Irwin *et al.*, 2016; Chaudhuri *et al.*, 2018; Kumar *et al.*, 2020). Some of the host factors are dispensable for the host but are essentially required for completion of virus life cycle. Since SB203580 was not shown to affect the viability of extracellular virus, the antiviral effect of SB203580 was due to inhibition of virus replication in the target cells, not simply due to inactivation of the extracellular virions. These host factors can serve as the targets for the antiviral drug development (Puschnik *et al.*, 2017; Gebre *et al.*, 2018; McDougall *et al.*, 2018). Since SB203580 targets a cellular factor (p38-MAPK), this should not have any tendency in inducing antiviral resistance.

CONCLUSION

A non-cytotoxic dose (8 µg/ml) of SB203580 resulted in reduction in SARS-CoV-2 yield, suggesting that SB203580 has potential to act as an antiviral agent against SARS-CoV-2.

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