In Vitro Resistance Profiling of S-892216, a Second Generation SARS-CoV-2 3CL^{pro} Inhibitor for the Treatment of COVID-19

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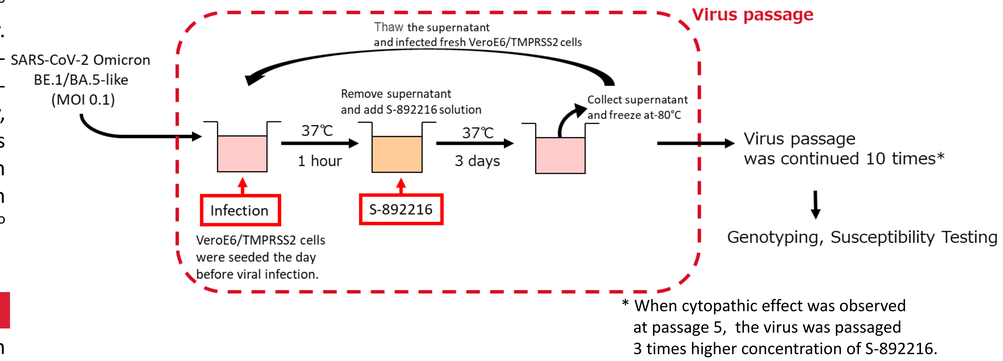
Background and Purpose

S-892216 is a candidate for the treatment of COVID-19 that has 3CL^{pro} inhibitory activity and antiviral activity. In this study, in vitro selection of SARS-CoV-2 with reduced susceptibility to S-892216 was conducted. Additionally, the antiviral activity of S-892216 was evaluated against SARS-CoV-2 with 3CL^{pro} substitution associated with reduced susceptibility to other 3CL^{pro} inhibitors, ensitrelvir and nirmatrelvir.

Methods

For the selection of SARS-CoV-2 with reduced susceptibility to S-892216 in vitro, VeroE6/TMPRSS2 cells infected with SARS-CoV-2 Omicron BE.1/BA.5-like variant (hCoV-19/Japan/TY41-702/2022) were cultured in the presence of S-892216 and passaged 10 times. Genotyping analysis of 3CL^{pro} and its cleavage sites in the isolated SARS-CoV-2 were then conducted. The antiviral activity of S-892216, ensitrelvir, and nirmatrelvir against the isolated and reverse genetics-derived SARS-CoV-2 (rgSARS-CoV-2) were assessed. rgSARS-CoV-2 with 3CL^{pro} substitutions including M49L and E166V were generated using circular polymerase extension reaction (CPER) system. The antiviral activity of S-892216 was assessed using rgSARS-CoV-2 infected VeroE6/TMPRSS2 cells.

Figure 1 S-892216 *in vitro* virus passage study



* When cytopathic effect was observed at passage 5, the virus was passaged 3 times higher concentration of S-892216.

Table 1 The genotyping and susceptibility testing results of isolated SARS-CoV-2 derived from S-892216 *in vitro* virus passage study

S-892216 (nmol/L)		Amino Acid Substitution		FC (vs EC_{50} of control virus)	
Passage 1 – 5	Passage 6 - 10	3CL^{pro}	Cleavage sites	S-892216	Nirmatrelvir
0	0	-	-	1.00	1.00
5.56	5.56	P252L L50F+P252L	-	3.51 – 3.84 4.80	1.18 – 1.56 1.52
5.56	16.7	L50F+P252L	-	7.04 – 10.2	2.61 – 3.25
16.7	16.7	M49K+P252L D48E+L50F+P252L M49K+N221K+P252L	-	23.7 10.0 28.7	0.812 2.46 2.16
16.7	50.0	M49K+P252L D48E+L50F+P252L	-	22.4 – 28.7 10.5	0.736 – 0.861 2.66
50.0	50.0	NT*1	NT*1	NT*1	NT*1

*1 Genotyping and susceptibility testing were not conducted because the virus titers were too low to conduct these tests.
NT: Not tested, FC: Fold change

Table 2 Fold change of S-892216, ensitrelvir, and nirmatrelvir against rgSARS-CoV-2 with 3CL^{pro} substitutions S-892216 *in vitro* virus passage study

	FC (vs EC_{50} of wild type)		
	S-892216	Ensitrelvir	Nirmatrelvir
D48E	2.12	1.25	1.08
M49R*1	13.7	2.19	0.820
M49K	3.66	1.57	0.389
L50F	1.51	1.18	1.34
N221K	1.76	1.30	1.19
P252L	1.22	1.08	1.15
M49K+P252L	4.24	1.46	0.457
L50F+P252L	3.25	1.58	2.02
D48E+L50F+P252L	5.82	2.01	2.35
M49K+N221K+P252L	5.18	1.60	0.630

*1 rgSARS-CoV-2 with M49R was constructed because lysine is also belonged to basic amino acid.

Table 3 Fold change of S-892216, ensitrelvir, and nirmatrelvir against rgSARS-CoV-2 with 3CL^{pro} substitutions associated with ensitrelvir and nirmatrelvir reduced susceptibility

	FC (vs EC_{50} of wild type)		
	S-892216	Ensitrelvir	Nirmatrelvir
T21I	1.67	1.11	1.82
D48G	1.50	4.92	1.45
M49L	0.420	33.0	0.833
P52S	0.384	4.41	0.648
S144A	0.697	8.24	1.40
M49L+S144A	0.661	124	1.63
T21I+E166V	0.276	2.76	36.0
L50F+E166V	0.405	2.81	43.4

Results

Fold change (FC) values of ensitrelvir and nirmatrelvir against the rgSARS-CoV-2 with isolated 3CL^{pro} substitutions were 1.08- to 2.01- fold for ensitrelvir and 0.630- to 2.35- for nirmatrelvir. The FC values of each compound against the isolated SARS-CoV-2 were 3.51- to 28.7-fold for S-892216 and 0.736- to 3.25- fold for nirmatrelvir.
The FC values of S-892216 against rgSARS-CoV-2 with 3CL^{pro} substitutions M49L and E166V were < 2-fold. SARS-CoV-2 with 3CL^{pro} substitutions of D48E, M49K, L50F, N221K, and P252L were isolated from the in vitro virus selection.

Conclusions

The isolated SARS-CoV-2- strains with reduced susceptibility to S-892216 carried 3CL^{pro} mutation - indicating that the target is 3CL^{pro}, did not show cross-resistance to ensitrelvir and nirmatrelvir. Furthermore, S-892216 did not show cross-resistance against nirmatrelvir and ensitrelvir-reduced susceptibility strains. These data suggest S-892216 might be effective if described ensitrelvir- and nirmatrelvir-resistant SARS-CoV-2 variants become relevant in the clinical setting.

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COI disclosure

Authors are employees of Shionogi & Co., Ltd. or Shionogi TechnoAdvance Research, Co., Ltd. Some authors are shareholder of Shionogi & Co., Ltd.

Reference

1) S Torii et al., *Cell Rep.* 2021; 35(3):109014.