Prophylactic effect of ensitrelvir in mice infected with SARS-CoV-2

Haruaki Nobori[1], Keiko Baba[1], Keita Fukao[1], Takayuki Kuroda[1], Kaoru Baba[2], Kazumi Matsumoto[2], Shinpei Yoshida[1], Ryosuke Watari[1], Takao Shishido[1], Teruhisa Kato[1]

1. Shionogi & Co., Ltd., 2 Shionogi TechnoAdvance Research & Co., Ltd.

SHIONOGI

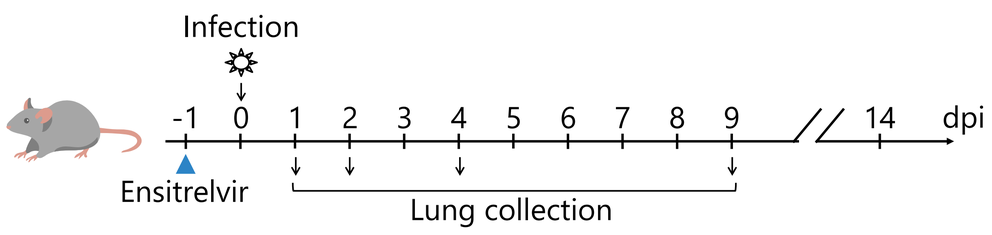
COI: Authors are employees of Shionogi & Co., Ltd. or Shionogi TechnoAdvance Research, Co., Ltd. Some authors are shareholder of Shionogi & Co., Ltd.

Background

The novel coronavirus infection (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is expected to continue as an endemic infection. It has been reported that the possibility of secondary infection of SARS-CoV-2 among cohousing family members is high, and SARS-CoV-2 prophylaxis for close contacts is a major unmet need. In this study, in a mouse SARS-CoV-2 lethal infection model, we verified the effect of prophylactic administration of ensitrelvir, a 3CL protease inhibitor, on the progression of disease after SARS-CoV-2 infection, and also predicted the plasma concentration that shows prophylactic effect in clinical setting.

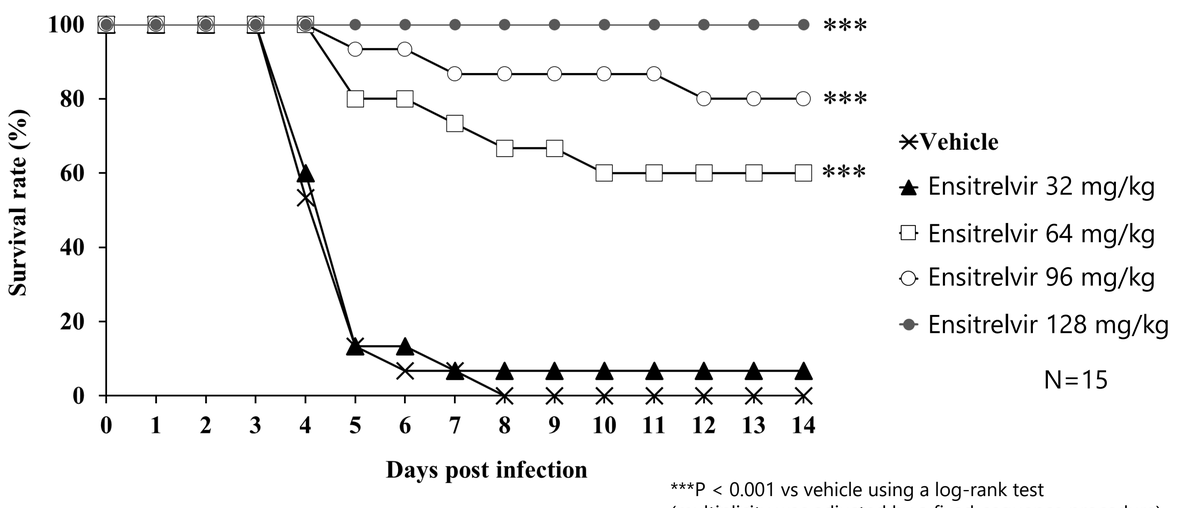
Method

BALB/c mice were prophylactically administered with a single dose of ensitrelvir (free form), suspended with 0.5% methylcellulose, subcutaneously (sc), followed by infection with SARS-CoV-2 mouse-adapted strain (MA-P10) 24 hours after administration. Body weight change and survival rate were evaluated. Lungs were collected 1, 2, 4, and 9 days after infection, and the lung virus titres were measured. In addition, plasma concentrations of ensitrelvir were determined.



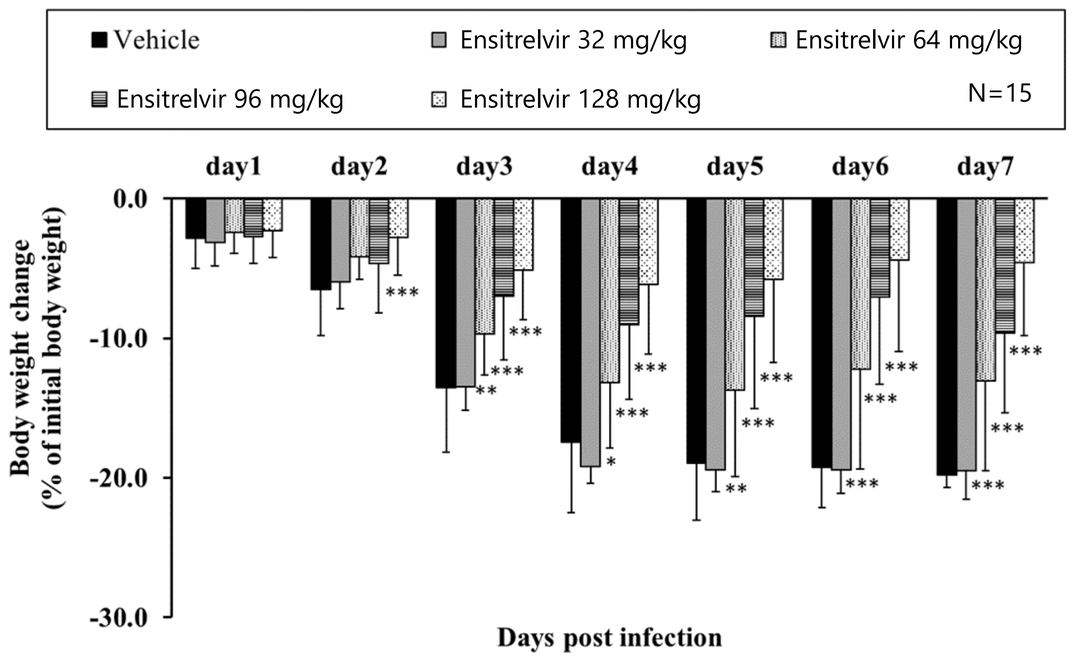
- **Animal:** Mice (female, Balb/c, 36-57 weeks)
- **Virus:** SARS-CoV-2 MA-P10 (mouse-adapted strain) 3×10^2 $TCID_{50}$/mouse
- **Ensitrelvir**: Suspended with 0.5% methylcellulose
- **Dosing:** subcutaneously, 1 day prior to infection, one shot

Figure 2. Effect of prophylactic treatment with ensitrelvir on mortality induced by infection with SARS-CoV-2 in mice



***P < 0.001 vs vehicle using a log-rank test
(multiplicity was adjusted by a fixed-sequence procedure)

Figure 3. Effect of prophylactic treatment with ensitrelvir on body weight change in SARS-CoV-2 infected mice

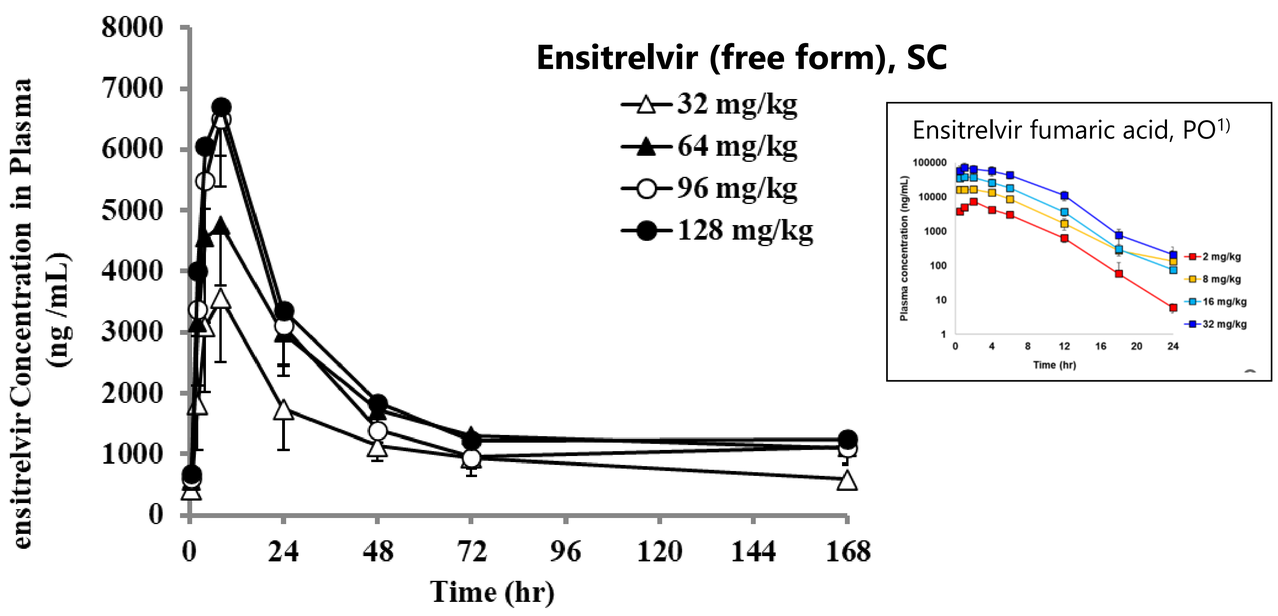


Each symbol represents the mean and SD in each group.
*P < 0.05, **P < 0.01, and ***P < 0.001 vs vehicle using a one-way analysis of variance model with equal variance assumption including the fixed effect of group and contrast method (multiplicity was adjusted by a fixed-sequence procedure)

Results

After a single subcutaneous administration of ensitrelvir, the plasma concentration reached a maximum at 8 hours after which it gradually decreased. Mean plasma concentrations 24 hours after administration of 32, 64, 96, and 128 mg/kg of ensitrelvir were 1,740, 2,990, 3,110, and 3,370 ng/mL, respectively. In ensitrelvir 64, 96, and 128 mg/kg administration groups, a significant suppressive effect on lethality was confirmed compared to the vehicle administration group, and body weight loss after infection was also significantly suppressed. In addition, the viral load in the lungs of mice were significantly suppressed in ensitrelvir 64 mg/kg or higher administration groups compared to the vehicle administration group.

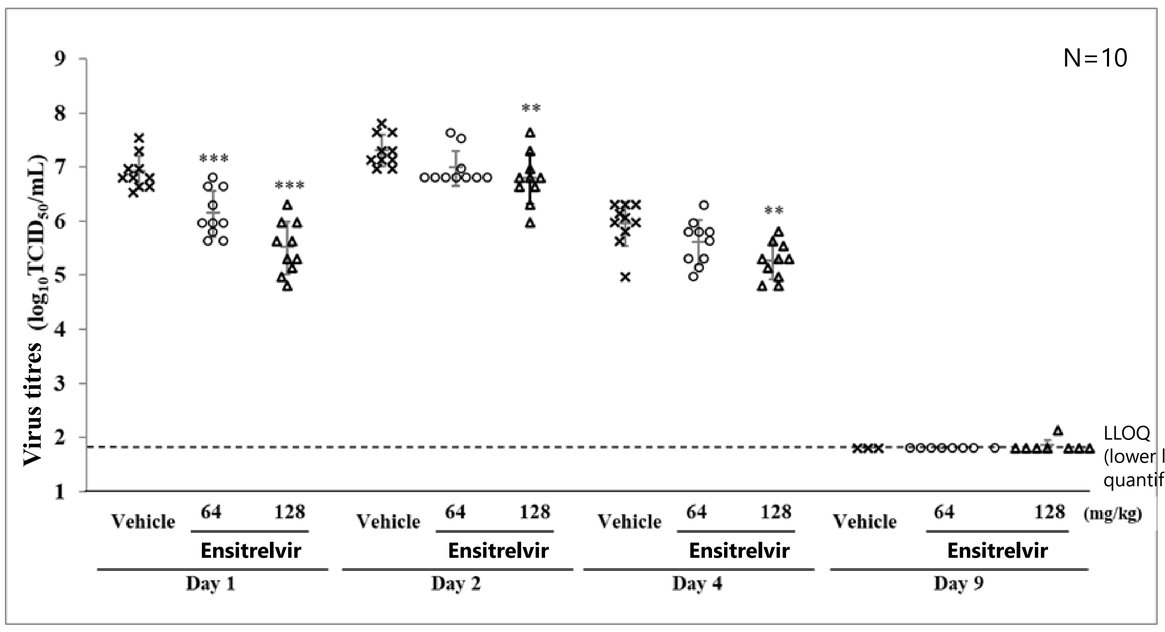
Figure 1. Plasma concentration of ensitrelvir over time in mice after single subcutaneous administration



	Mouse, SC				Mouse, PO	Human, PO*
	32 mg/kg	64 mg/kg	96 mg/kg	128 mg/kg	32 mg/kg	375/125 mg**
C_{24h} (ng/mL)	1,740 ± 670	2,990 ± 550	3,110 ± 820	3,370 ± 900	210 ± 67	3,970 (Day 10) 2,980 (Day 11)
$T_{1/2}$ (h)	-	218	-	-	2.09	57.6[2)]

Data are expressed as the mean ± SD of mice in each group (N=4).
*Healthy White male
**once-daily doses of ensitrelvir 375 mg on day 1 and 125 mg on days 2 to 5

Figure 4. Effect of prophylactic treatment with ensitrelvir on the viral titres in lungs in SARS-CoV-2 infected mice



Each symbol represents the mean and SD in each group.
**P < 0.01 and ***P < 0.001 vs vehicle calculated using Dunnett's method

Conclusion

According to data for reduced mortality, suppression of body weight loss, and viral load in lungs of mice treated with ensitrelvir in this study, ensitrelvir was effective for prophylaxis in mice at a dose of 64 mg/kg or greater, that achieve plasma concentration of 2,990 ng/mL at the time of viral infection. Based on the result in mice, we designed a clinical trial for prophylactic effect in human. The prophylactic effect against SARS-CoV-2 infection could be expected up to 10-11 days post first administration in human (orally once daily 375 mg on Day 0, 125 mg on Days 1 to 4).

Acknowledgment

We acknowledge Prof. Hirofumi Sawa (Hokkaido University, Sapporo, Japan) for providing the mouse-adapted SARS-CoV-2 (MA-P10) strain.

Reference

1. Unoh et al. *J. Med. Chem.* 2022 May 12; 65 (9), 6499–6512
2. Shimizu et al. JAID west 2022 (P-055)