P0429

Hamster model to evaluate post-COVID-19 condition treated with ensitrelvir: smell disorder

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Background

Ensitrelvir, an oral SARS-CoV-2 3CL protease inhibitor for treatment of COVID-19, has received approval in Japan in March 2024. In clinical study, early administration of ensitrelvir has been reported to be effective in suppressing the onset of symptoms for post-COVID-19 condition such as smell disorder (Tsuge Y, et al. IDWeek 2023 Poster 549, Fukushi A, et al. ESWI 2023 Poster 681). However, the pathogenesis of these symptoms and the mechanism of the symptom suppression by ensitrelvir are not clearly understood. In this study, we explored a post-COVID-19 condition model by measuring grip strength and loss of smell in hamsters four weeks after SARS-CoV-2 infection and evaluated the efficacy of ensitrelvir against smell disorder.

Methods

Six-weeks old male Syrian hamsters were intranasally inoculated with 10000 $TCID_{50}$/hamster SARS-CoV-2 Delta strain (hCoV-19/Japan/TY11-927/2021). From one day after infection, the hamsters were administered 50 mg/kg ensitrelvir fumaric acid in 0.5% MC (PO), or 750 mg/kg initial dose/250 subsequent (750/250 mg/kg) nirmatrelvir in 0.5% MC (SC) and 50 mg/kg ritonavir in 0.5% MC (PO) twice daily for five days (Kuroda et al., *J Antimicrob Chemother*, 2023). Four weeks post infection (wpi), grip force test and smell test were conducted. Grip force was calculated by dividing the grip strength by individual body weight. Smell test using 2,4,5-trimethyl thiazoline (TMT), an olfactory repellent for rodents, was conducted by measuring escape behaviour. After behavioural tests, lungs, nasal turbinates (NT), olfactory bulbs (OB), and hippocampus (HPC) were collected. Viral titres were measured by $TCID_{50}$ assay and mRNA expression of virus RNA and inflammatory markers was quantified by RT-qPCR.

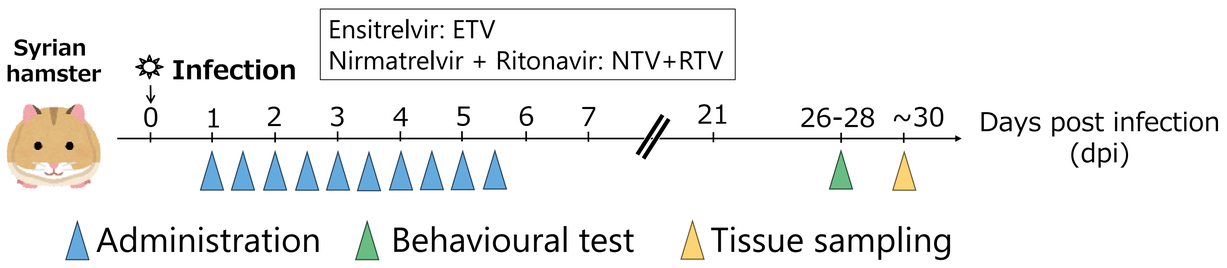
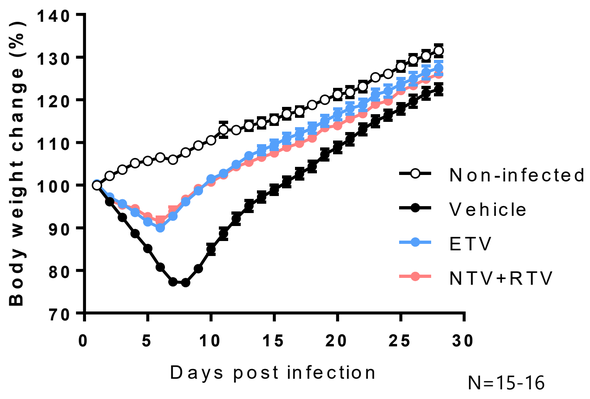
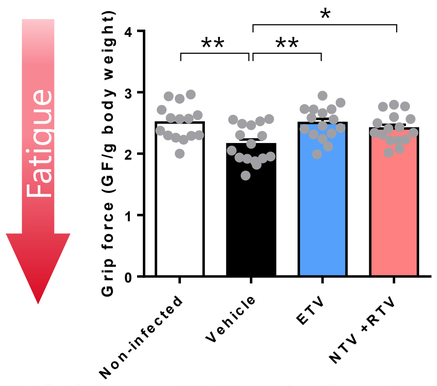


Figure 1a Body weight changes



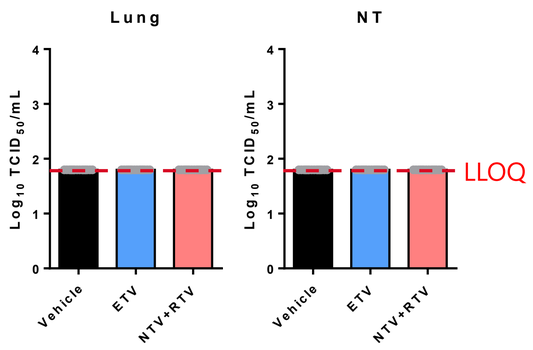
N=15-16

Figure 1b Grip force test 4 wpi



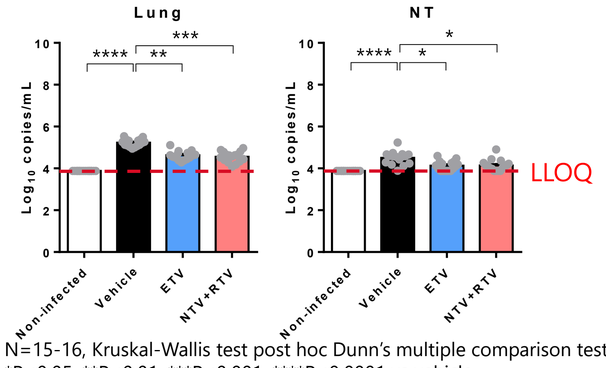
N=15-16, One-way ANOVA post hoc Dunnett's multiple comparisons test, *P<0.05, **P<0.01 vs vehicle

Figure 1c Viral titres in lung and NT 4 wpi



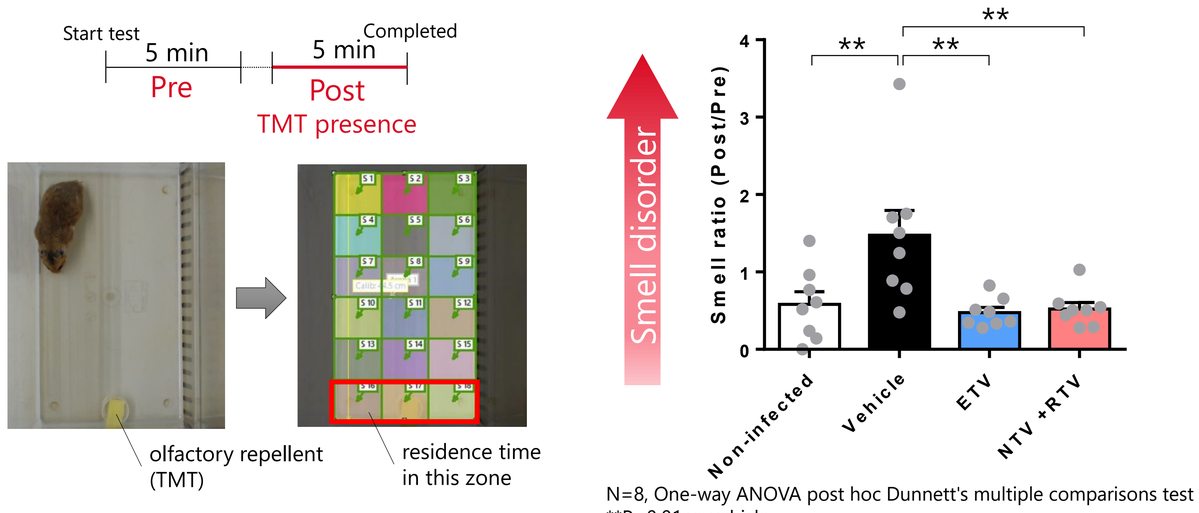
N=15-16, LLOQ: Lower limit of quantification

Figure 1d Viral RNA loads in lung and NT 4 wpi



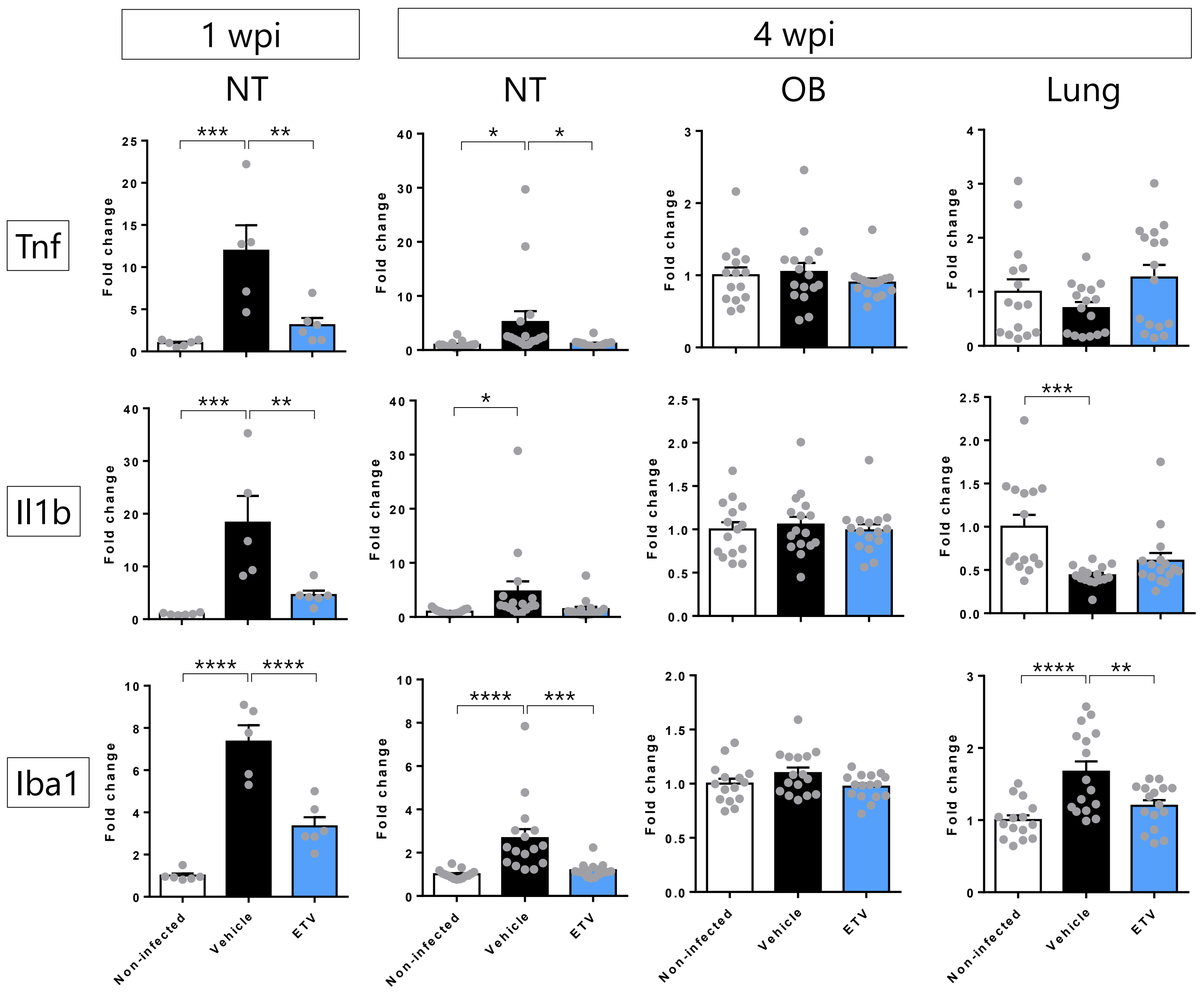
N=15-16, Kruskal-Wallis test post hoc Dunn's multiple comparison test
*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs vehicle
LLOQ: Lower limit of quantification

Figure 2 Schematic diagram of analysis method and the result of smell test 4wpi



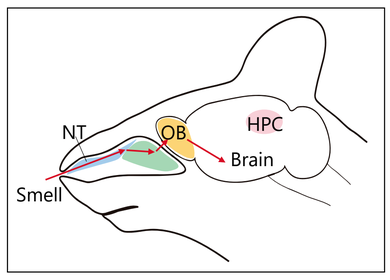
N=8, One-way ANOVA post hoc Dunnett's multiple comparisons test
**P<0.01 vs vehicle

Figure 3 Quantifying expression of Tnf, Il1b and Iba1 in NT, OB and Lung by RT-qPCR



N=8-16, One-way ANOVA post hoc Dunnett's multiple comparisons test, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs vehicle

Figure 4 Schematic diagram of the olfactory pathway and heatmap showed the correlation between smell ratio and Inflammatory marker mRNA levels 4 wpi



	Tnf	Il1b	Il6	Cxcl10	Iba1	Tgfb	Ccl2	Cd3d	Cd8a	Nefl	Ccl11	Ccl5
NT	**	**		**	***	**	*	**	****	*	*	***
OB												*
HPC			*				**	**	*			
Lung		*			**	*		*			****	

1 / 0 / -1

Including non-infected, vehicle, and ETV groups, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, Pearson's correlation coefficient

Results

Four weeks after infection, SARS-CoV-2 virus titres in lungs and NT were below the lower limit of quantification (Fig.1c). ETV or NTV+RTV treatment caused early recovery of body weight and suppression of virus RNA, and improvement of grip force and sense of smell 4 wpi (Fig. 1a, b, d and 2). The mRNA expression levels of inflammatory markers scarcely increased in lungs and OB due to infection (Fig. 3). In contrast, that levels of the inflammatory markers significantly increased in NT from 1 to 4 wpi and many of them were suppressed by ensitrelvir treatment (Fig. 3). Significant positive correlations were observed with many inflammation-related markers in NT unlike other tissues (Fig. 4).

Conclusion

The infected hamsters without antiviral treatment recovered their weight by 4 weeks after infection, however they showed a decline in motor functions and smell disorder in the absence of infectious virus, and ensitrelvir suppress decline in motor functions. This study suggested that chronic inflammation may occur in NT over a long period of time after SARS-CoV-2 infection, leading to smell disorder in the hamster model. We demonstrated that early ensitrelvir treatment after infection suppressed inflammation in the NT and prevented the onset of smell disorder.

Acknowledgement

SARS-CoV-2 was kindly gifted from National Institute of Infectious Diseases (NIID).