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LETTER TO THE EDITOR

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5-Aminolevulinic acid antiviral efficacy against SARS-CoV-2 omicron variant in vitro

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Abstract

The coronavirus disease 2019 (COVID 19) pandemic continues to pose a threat to global health. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant (B.1.1.529) has spread rapidly worldwide and became dominant in many countries. A natural 5-aminolevulinic acid (5-ALA) with sodium ferrous citrate (SFC) has demonstrated antiviral activity in Wuhan, Alpha, Beta, Gamma, and Delta variants of SARS-CoV-2 infections in vitro. In this study, we report antiviral activity of 5-ALA, 5-ALA with SFC led to IC_{50} of 329 and 765/191, respectively after infection with Omicron variant of SARS-CoV-2 in vitro. Our finding suggests that 5-ALA could be used as antiviral drug candidate to treat Omicron variant infected patients.

Keywords: SARS-CoV-2, Omicron variant, 5-ALA, SFC, Antiviral activity

To the Editor,

Multiple severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants have emerged a year and a half from the onset of coronavirus disease 2019 (COVID-19) pandemic. As of February 2022, Omicron variants have been divided into four distinct sub lineages: BA.1, BA.1.1, BA.2, and BA.3 [1–3]. The number of Omicron variant cases has increased in many regions of the world, spreading more easily than previously described SARS-CoV-2 isolates [4]. The Omicron variant has substantial spike protein mutations and is able to escape immune protection elicited by both vaccine and previous infection [5, 6]. Several direct-acting antivirals against COVID-19 have been approved or are under clinical development and can be divided in two categories; small molecules interfering with virus replication machinery, and monoclonal antibodies directed against the spike protein [7]. Aside from improving vaccinations against Omicron and future variants, we must develop new antiviral drugs [8, 9]. Antiviral drugs including remdesivir, molnupiravir, and nirmatrelvir inhibited SARS-CoV-2 Omicron variant infection [7, 10]. A natural amino acid, 5-aminolevulinic acid (5 ALA), is produced from most animals and plants which are present in food. In our previous studies, we reported antiviral activity of 5-ALA with or without sodium ferrous citrate (SFC) against the SARS-CoV-2 Wuhan strain and its variants including Alpha, Beta, Gamma and Delta strains [11, 12]. In this study, we evaluated the antiviral effect of 5-ALA with or without SFC against the SARS-CoV-2 Omicron variant in vitro. Vero E6 cells were treated with remdesivir or 5-ALA with or without SFC for 72 h (h) and then infected with SARS-CoV-2 Omicron variant (TY38-873, BA.1, provided by the Japan National Institute of Infectious Diseases) at a multiplicity of infection of 0.02. After 48 h post infection,

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the infected cell supernatants were harvested for viral RNA extraction [12, 13]. The SARS-CoV-2 antiviral assay is based on previously established specific quantitative real time PCR (qRT-PCR) [12] using cell supernatant RNA. The antiviral drug effect of remdesivir against the Omicron variant showed an IC_{50} (virus inhibition by 50%) of 0.3 μM (Fig. 1A). 5-ALA, 5-ALA and SFC inhibited SARS-CoV-2 Omicron variant infection in a dose dependent manner with an IC_{50} of 329 and 765/191, respectively in vitro (Fig. 1B, C, Table 1). A cell viability assay was conducted in parallel with the antiviral assay [12, 13] and no cytotoxic effects were observed with CC_{50} (cell survival by 50%) of 5-ALA > 2000 μM and of SFC > 500 μM in Vero E6 cells (Table 1). The Omicron variant which has notable mutations in the receptor binding domain of spike glycoprotein appears to be highly transmissible and less responsive to several of the currently used drugs [14]. Exogenously supplied 5-ALA prompted increased generation of protoporphylin IX (PPIX) and heme inside host cells, likely interfering with interaction of G-quadruplex (G4) structures [15] which inhibited SARS-CoV-2 infection. The G4 structure included in coronaviruses plays a key role in the genome replication/transcription [16]. 5-ALA with SFC is a supplement formulation registered in Japan as a food with functional claims. In a recent clinical study, Japanese patients with COVID-19 who were given 5-ALA and SFC capsules orally experienced a shorter time to recovery than that reported for patients who received only standard care for SARS-CoV-2 infection [17]. Recruitment for clinical trials on the effects of 5-ALA with SFC on COVID-19 outcomes in humans has been completed and the data is now being analyzed (Japan Registry of Clinical Trials CRB 7180001 and 3190006, respectively). Mitochondrial dysfunction has been reported as a cause of disorders in COVID-19 [18]. Given that 5-ALA activates the respiratory chain of mitochondria via heme bio-synthesis, maintenance of mitochondrial function is also expected to play a role in the effect of 5-ALA on the prevention and treatment of COVID and long COVID. In conclusion, we report the antiviral effects of 5-ALA with or without SFC on SARS-CoV-2 Omicron variant in vitro as a potential therapeutic and prophylaxis for COVID-19.

Table 1 IC_{50} and CC_{50} values of 5-ALA and 5-ALA with SFC against SARS-CoV-2 Omicron variant

SARS-CoV-2 variants*	Compound	IC_{50} (μM)	CC_{50} (μM)
Wuhan	5-ALA	207	> 2000
	5-ALA/SFC	235/58.7	> 2000/ > 500
Alpha	5-ALA	104	> 2000
	5-ALA/SFC	173/43.2	> 2000/ > 500
Beta	5-ALA	1592	> 2000
	5-ALA/SFC	1311/327.7	> 2000/ > 500
Gamma	5-ALA	> 2000	> 2000
	5-ALA/SFC	1516/379	> 2000/ > 500
Delta	5-ALA	> 2000	> 2000
	5-ALA/SFC	397/99.2	> 2000/ > 500
Omicron	**5-ALA**	**329**	**> 2000**
	5-ALA/SFC	**765/191**	**> 2000/ > 500**

IC_{50} and CC_{50} of SARS-CoV-2* variants (Wuhan, Alpha, Beta, Gamma and Delta) was shown in our previous study

In 5-ALA/SFC compound, the ratio of 5-ALA to SFC was fixed as 4:1

IC_{50} 50% inhibition concentration, *CC_{50}* 50% cytotoxicity concentration, *5-ALA* 5-aminolevulinic acid, *SFC* sodium ferrous citrate

Bold text indicated IC_{50} and CC_{50} of Omicron variant in this study

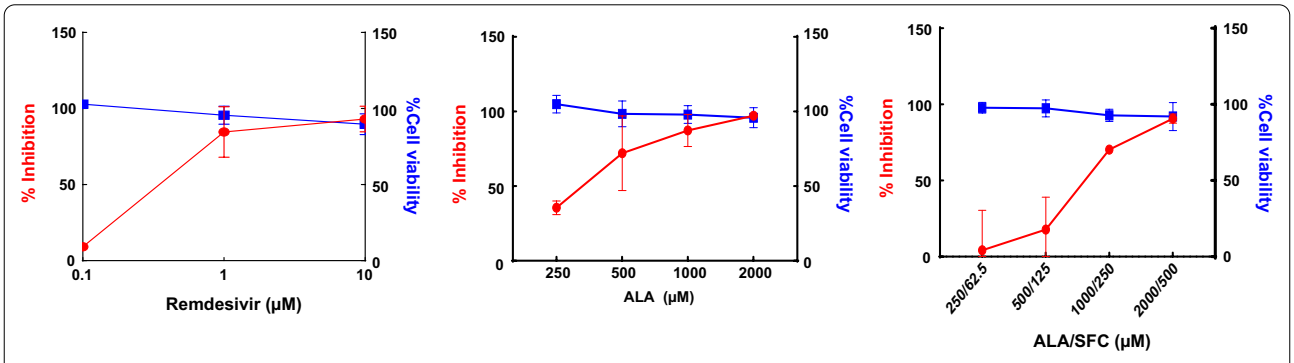

Fig. 1 Antiviral effect of remdesivir (**A**) 5-ALA (**B**) and 5ALA with SFC (**C**) against SARS-CoV-2 Omicron variant. Vero E6 cells were pretreated with remdesivir, ALA with and without SFC for 72 h and challenged with SARS-CoV-2. Infected cell supernatants at 48 h pi (MOI 0.02) were quantified by quantitative real time RT-PCR assay. The blue and red lines represent the CC_{50} and IC_{50}, respectively; the blue squares represent cell viability (%) and the red circles represent SARS-CoV-2 infection inhibition (%). Data were collected from at least two independent experiments (in replicate) and shown data correspond to the mean ± SD

Abbreviations
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; 5-ALA: 5-Aminolevulinic acid; SFC: Sodium ferrous citrate; PPIX: Protoporphyrin IX; qRT-PCR: Quantitative real time reverse-transcription polymerase chain reaction; IC_{50}: Virus inhibition by 50%; CC_{50}: Cell survival by 50%; G4: G-quadruplex.

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Author contributions
MMNT, JY, KK, KM: conceptualization, study design. MMNT, TS, YS, YK, DI: data analysis and investigation. MMNT: writing-original draft preparation. MMNT, NS, CS, YS, YK, KK: writing-review and editing. JY, KK, KM: supervision and funding acquisition. All authors read and agreed final version of the manuscript.

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Declarations

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Not applicable.

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Competing interests
Kita K. is a Scientific Advisor of Neopharma Japan. The other authors declare no competing interests.

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