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Coronavirus disease 2019 (COVID-19) has affected millions of people around the world and has resulted in more than a million deaths. It is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel zoonotic coronavirus that was first reported in Wuhan, Hubei province, China, in December 2019 [1]. The main protease (Mpro) of SARS-CoV-2, also called as 3CLpro, is an important therapeutic target due to its important role in the processing and maturation of the viral polyprotein [2,3]. GC376 is a pre-clinical dipeptide-based protease inhibitor that has been previously used for managing feline infectious peritonitis virus (FIPV). Since both GC373 and GC376 have already been successfully used in treating animal coronavirus infection, they can be considered as strong drug candidates for COVID-19 in humans. GC376 is a broad-spectrum antiviral drug that inhibits Mpro of several viruses, including the coronaviruses like feline coronavirus, porcine epidemic diarrhoea virus, severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus, ferret, and mink coronavirus. However, further studies should be conducted to evaluate the potency, efficacy, and safety of these broad-spectrum Mpro inhibitors in patients with COVID-19. The lessons learned from the successful use of drug candidates for treating animal coronavirus infections will help us to develop framework for their use in human trials.

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Protease inhibitor GC376 for COVID-19: Lessons learned from feline infectious peritonitis

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**ABSTRACT**

The main protease (Mpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an important therapeutic target as it plays a major role in the processing and maturation of the viral polyprotein. GC376 is a pre-clinical dipeptide-based protease inhibitor that has been previously used for managing feline infectious peritonitis virus (FIPV). Since both GC373 and GC376 have already been successfully used in treating animal coronavirus infection, they can be considered as strong drug candidates for COVID-19 in humans. GC376 is a broad-spectrum antiviral drug that inhibits Mpro of several viruses, including the coronaviruses like feline coronavirus, porcine epidemic diarrhoea virus, severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus, ferret, and mink coronavirus. However, further studies should be conducted to evaluate the potency, efficacy, and safety of these broad-spectrum Mpro inhibitors in patients with COVID-19. The lessons learned from the successful use of drug candidates for treating animal coronavirus infections will help us to develop framework for their use in human trials.

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useful to manage COVID-19.

The safety and efficacy of GC376, a dipeptide-based protease inhibitor was previously evaluated on client-owned cats with FIP, where it has showed promising therapeutic efficacy, particularly in the cats with certain presentations of FIP [4]. It also inhibits SARS-CoV-2 in Vero cells by targeting the catalytically active sites of Mpro [2], and has antiviral activity against SARS-CoV-2 at an EC_{50} value of 3.37 µM [10]. In addition, it acts against SARS-CoV and MERS-CoV, the other two zoonotic coronaviruses infecting human beings [3,11]. Studies have also shown that it can inhibit the main protease of ferret and mink coronavirus [12]. Therefore, GC376 can be considered as a broad-spectrum antiviral drug that inhibits Mpro of several viruses, including the coronaviruses like FCoV, porcine epidemic diarrhoea virus (PEDV), SARS-CoV, MERS-CoV, SARS-CoV-2, ferret, and mink coronavirus [3,5,11,12]. This may be because of the highly conserved structure of Mpro among these viruses [5,12].

GC376 is the prodrug of GC373, another dipeptide-based protease inhibitor. In addition to being the prodrug, GC373 was also reported to effectively inhibit the Mpro of SARS-CoV-2 with an IC_{50} value in the nanomolar range [5]. The ability of GC373 and GC376 to inhibit SARS-CoV-2 was evaluated with plaque reduction assays using infected Vero E6 cells. The findings indicate that both the drugs are efficient inhibitors of SARS-CoV-2 with high therapeutic index (>200) [5]. Based on the available data, both GC373 and GC376 can be advanced quickly into the next stage of evaluation that includes human trials.

Although the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 has proof-reading function, the virus mutates, leading to drug resistance [13]. SARS-CoV Mpro inhibitors can be used either alone or in combination with viral RdRp-inhibitors, to achieve synergistic antiviral activity and to suppress the emergence of drug resistance [10]. The combined use of GC376 with Remdesivir, a nucleotide analogue that inhibits RdRp of SARS-CoV-2, produces additive effect, thereby enhancing the overall antiviral activity [2]. Remdesivir monotherapy has raised concerns regarding the possible development of drug resistance [13]. Therefore, the addition of SARS-CoV-2 Mpro inhibitors, such as GC376, into the treatment regimen will ensure that SARS-CoV-2 infection is controlled at multiple levels (Fig. 2).

Treatment of FIP in cats with GC376 was associated with side effects such as transient stinging at the injection sites, subcutaneous fibrosis, hair loss, and abnormal eruption of permanent teeth in juvenile cats [4]. Therefore, further studies are required to evaluate the possible side effects associated with the use of GC376 in animal models, before its use in clinical trials. Considering its potentials for side effects, GC376 should only be used for a short-period (1–2 weeks) to treat COVID-19 [2].

Conclusion and future perspectives

SARS-CoV-2 Mpro is an important drug target as it plays an essential role in the cleavage of viral polyproteins. Repurposed antiviral drugs, especially the protease inhibitors can be considered as an important therapeutic strategy for managing COVID-19. Since both GC373 and GC376 have already been successfully used in treating animal coronavirus infection, they can be considered as strong drug candidates for COVID-19 in humans. Both the drugs inhibit the replication of SARS-CoV-2 in cell cultures by inhibiting Mpro. Therefore, further studies can be conducted to evaluate the potency, efficacy, and safety of these broad-spectrum SARS-CoV-2 Mpro inhibitors in patients with COVID-19. Over the years, researchers have tried different strategies to develop or identify suitable therapeutic candidates against FIPV. The experience they have gained through these studies is now becoming fruitful in identifying therapeutic drugs for COVID-19. The lessons learned from the successful use of drug candidates for treating animal coronavirus infections will help us to develop framework for their use in human trials. Furthermore, understanding the mutation that give rise to virulent and lethal FIPV will provide an insight into the relationship between different strains of SARS-CoV-2 and their virulence.

Fig. 1. Neurological FIP in a cat with CNS involvement presented with neurological deficits that was treated with GS-441524. Reproduced from Dickinson (2020) Creative Commons Attribution License (CC BY). A–D: pre-contrast, pre-treatment MRI sequences. E–H: post-contrast T1-weighted and fluid-attenuated inversion recovery MRI sequences showing multifocal leptomeningal lesions (arrowheads). I–L: treatment with GS-441524 (10 mg/kg) resulted in resolution of clinical signs and MR lesions in images acquired 7.5 months after initiation of treatment. (T1 - T1-weighted, FL - fluid-attenuated inversion recovery, +C - using contrast: gadopentetate dimeglumine).
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Fig. 2. Therapeutic candidates that can inhibit the replication of SARS-CoV-2 by inhibiting the main protease (M^{pro}) (also called as 3CL^{pro}) and RdRp.
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