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LETTER TO THE EDITOR

25-Hydroxycholesterol is a potent SARS-CoV-2 inhibitor

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Dear Editor,

As of July, 2020, the ongoing pandemic of coronavirus diseases 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously 2019-nCoV) has caused more than 10.3 million human infections, with more than 506,000 deaths worldwide according to the World Health Organization. The clinical manifestations of COVID-19 vary from no asymptomatic infection, mild "flu-like" symptoms, to lethal acute respiratory distress syndrome. The case mortality and fatality rates in people infected with SARS-CoV-2 increase steeply with age, and fatal outcomes are almost exclusively seen in people older than 50 years.[1] Although the Food and Drug Administration has authorized emergency use of remdesivir for COVID-19 treatment in the US, the need for safe and effective antiviral drugs against SARS-CoV-2 remains urgent and unmet.

25-hydroxycholesterol (25HC) is the product of cholesterol oxidation by the enzyme cholesterol-25-hydroxylase, encoded by the gene CH25H.[2] In the innate immune system, CH25H expression is induced in macrophages and dendritic cells in response to various toll-like receptor ligands and interferon (IFN).[3] 25HC, the enzymatic product of CH25H, is natural oxysterol and can control sterol biosynthesis by regulating sterol-responsive element binding proteins (SREBPs).[4,5] Recently, the metabolomic and lipidomic alterations in COVD-19 patients sera have been reported, and 7-hydroxycholesterol, the analogue of 25HC, was up-regulated in COVID-19 patients' sera.[6] Additionally, Wu et al. found the metabolomic and lipidomic were changed in COVID-19 patients' sera and proposed the plasma biomarkers associated with COVID-19 would be potential therapeutic targets.[7] However, whether 25HC is involved in COVID-19 pathogenesis and plays antiviral roles remain not determined.

In the present study, we continuously monitored the clinical development and 25HC kinetics in the serum of a 73-year-old female COVID-19 patient from Shiyan, Hubei province (Fig. 1a). The patient presented with dysphasia on February 7, 2020, and was admitted to hospital on February 15 after experiencing bleeding in stool. Lung X-ray computed tomography showed ground-glass opacity. The patient had many underlying conditions including hypertension, coronary heart disease, diabetes and sicca syndrome, and she was transferred to intensive care unit (ICU) on February 17. The throat swab and sputum sample were subjected to SARS-CoV-2-specific reverse transcription quantitative PCR (RT-qPCR) detection, and positive results were reported on February 18 and 19, respectively. Although IgG and IgM assay were negative, the patient was diagnosed as COVID-19. The patient lost consciousness on February 24 and died on February 28 (Fig. 1a). We obtained four serum samples from the patient at the indicated time points (Fig. 1a), and liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis showed that the serum concentration of 25HC remained stable in the first days, but suddenly ascended up to 58 ng/mL on February 26, two days before her death, which is far higher than the average of random healthy individuals (Fig. 1b). This sudden increase of 25HC in serum and its potential link to the clinical deterioration of COVID-19 patient inspired us to explore the role of 25HC during SARS-CoV-2 infection.

To recapitulate the increase of 25HC in COVID-19 patients, we further determined the serum concentration of 25HC in SARS-CoV-2-infected hACE2 mice.[8] All animals infected intranasally (i.n.) with SARS-CoV-2 had a higher serum concentration of 25HC than the control group (Fig. 1c). These results suggested that 25HC was elevated in response to SARS-CoV-2 infection.

Next, we sought to determine whether CH25H, the enzyme of 25HC, was induced by SARS-CoV-2 infection. As expected, human Caco-2 cells infected with SARS-CoV-2 showed a significant upregulation of *CH25H* gene as well as other ISGs including *IFN-β*, *MX1*, *ISG15* and *IFIT1* (Supplemental information, Fig. S1). Similarly, intranasal infection of SARS-CoV-2 mouse-adapted strain MASCp6 also led to the upregulated mRNA level of *CH25H* in the lung tissue from the infected mice compared with the that from the control animals (Fig. 1d). Most importantly, over-expression of CH25H resulted in a significant reduction in SARS-CoV-2 viral genome in Vero cells (Supplemental information, Fig. S2). Collectively, these in vitro and in vivo results further supported the antiviral role of 25HC during SARS-CoV-2 infection.

We next tested the cytotoxicity of 25HC in Vero cells by standard 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, 25HC had no obvious cytotoxicity at concentration as high as 400 μM, and the half cytotoxic concentration (CC_{50}) of 25HC was calculated as >400 μM (Supplemental information, Fig. S3). We then tested the antiviral activity of 25HC against SARS-CoV-2 infection in Vero cells. As expected, 25HC significantly inhibited SARS-CoV-2 replication in a dose-dependent manner, and the half-maximal effective concentration (EC_{50}) value of 25HC was calculated to 3.675 μM (Fig. 1e). Meanwhile, pretreatment with 25HC remarkably reduced viral protein production in SARS-CoV-2-infected Vero cells by western blot and immunofluorescence assay, respectively (Fig. 1f, g). Furthermore, the antiviral effect of 25HC was tested using a vesicular stomatitis virus (VSV)-based reporter pseudovirus that expresses the spike (S) protein of SARS-CoV-2.[9] Similar to results from authentic SARS-CoV-2 virus, 25HC exhibited direct inhibition on pseudovirus production, indicating that 25HC plays antiviral roles at the entry step (Fig. 1h).

Finally, we assayed the in vivo antiviral effect of 25HC in a newly established mouse model based on a SARS-CoV-2 strain MASCp6.[10] Upon intranasal infection with 2×10^4 50% tissue culture infectious dose ($TCID_{50}$) of MASCp6, all mice treated with vehicle sustained robust viral replication in the lung and trachea at 3 day-post-infection (dpi); intragastric administration with 100 mg/kg of 25HC daily significantly reduced viral RNA loads in both lung and trachea (Fig. 1i). Additionally, standard toxicity in rats demonstrated that intragastric administration with 25HC at 1000 mg/kg for 14 days didn't cause any obvious adverse effects, highlighting the safety profile of 25HC.

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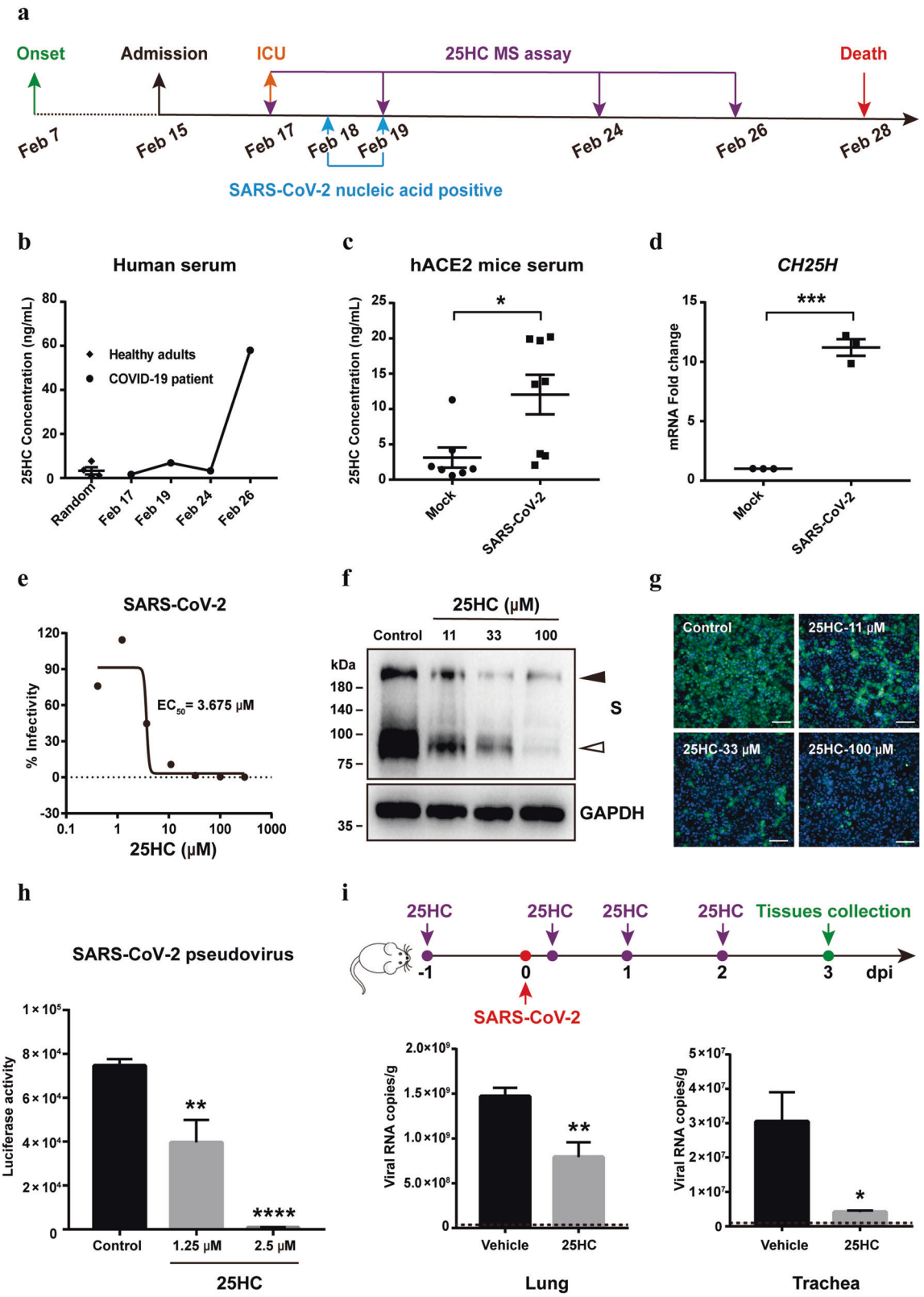

Previously, we and others have demonstrated that 25HC is a potent inhibitor for many pathogenic viruses with envelope, e.g., Ebola virus (EBOV), human immunodeficiency virus (HIV), Hepatitis C virus (HCV) and Zika virus (ZIKV).[2,3,11] The underlying mechanisms for its antiviral activity include suppressing fusion between viral and cell membranes, blocking membranous web formation, and inducing antiviral genes. The newly identified SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus that belongs to *betacoronavirus* genus together with the other two highly pathogenic

Fig. 1 25HC is upregulated upon SARS-CoV-2 infection and shows in vitro and in vivo antiviral activities against SARS-CoV-2. a Schematic diagram of the diagnosis and disease procession of the COVID-19 patient. **b** The serum concentration of 25HC in healthy adults and the COVID-19 patient were determined by MS. **c** Serum concentration of 25HC in SARS-CoV-2-infected hACE2 mice. The hACE2 mice were infected intranasally (i.n.) with 5×10^5 $TCID_{50}$ of SARS-CoV-2, and the serum concentrations of 25HC at 5 dpi were quantified by MS. Mock, $n = 7$; SARS-CoV-2, $n = 8$. **d** SARS-CoV-2 infection upregulated mRNA level of *CH25H* in mice lung. BALB/c mice were infected i.n. with the SARS-CoV-2 mouse-adapted strain MASCp6, and the mRNA level of *CH25H* in lung was tested by RT-qPCR at 3 dpi. **e** The antiviral activity of 25HC in vitro. Vero cells were treated with serial dilutions of 25HC and then infected with 20 $TCID_{50}$ of SARS-CoV-2. Viral RNA copies in supernatant were quantified by RT-qPCR at 48 h-post-infection (hpi). *Y*-axis represents the mean of percent infectivity normalized to the ethyl alcohol (EtOH) control group. **f** Western blotting of SARS-CoV-2-infected Vero cells that received 25HC treatment. Black arrow indicates bands corresponding to uncleaved S proteins (S0) and grey arrow indicates bands corresponding to the S2 subunit. **g** Immunofluorescence assay of SARS-CoV-2-infected Vero cells that received 25HC treatment. SARS-CoV-2 S protein was stained in green, and DAPI in bule. Scale bars, 100 μm. **h** SARS-CoV-2 pseudovirus assay. Huh 7 cells were treated with the indicated concentrations of 25HC, and then infected with SARS-CoV-2 pseudovirus at 2×10^4 $TCID_{50}$ for 24 h. Luciferase activity in cell lysates was determined and compared with the EtOH control. **i** The in vivo antiviral effect of 25HC against SARS-CoV-2. Mice were administrated intragastrically with 25HC (100 mg/kg) or vehicle for 24 h and subjected to intranasal challenge with the SARS-CoV-2 mouse-adapted strain MASCp6. Lung and trachea viral loads were tested by RT-qPCR at 3 dpi. Vehicle/25HC, $n = 3$. Dash lines denote the detection limit. All data are shown as means ± SEM, $^*P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$, $^{****}P \le 0.0001$, with unpaired Student's *t*-test.

pathogens, SARS-CoV and Middle East Respiratory Syndrome coronavirus (MERS-CoV). In the present report, we firstly demonstrate that 25HC can prevent SARS-CoV-2 infection at the entry step, and its antiviral effects against other human coronaviruses can be expected.

In conclusion, our present study shows that 25HC was elevated in a fatal COVID-19 patient as well as in SARS-CoV-2-infected hACE2 mice. The potential of 25HC serum concentration as a risk marker for severe illness of COVID-19 deserve further investigation. More importantly, 25HC shows potent in vitro and in vivo antiviral effect on SARS-CoV-2 infection. The EC_{50} of 25HC was comparable to other validated drugs including chloroquine and hydroxychloroquine.[12,13] As a natural metabolite of human, the ideal safety profile, as well as the antiviral potency shown in this study, support its further clinical development for COVID-19 treatment.

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AUTHOR CONTRIBUTIONS

C.-F.Q., G.C. and H.Y. conceived and designed the experiments. S.Z., Y.-Q.D. and C.Z. performed the majority of the experiments and analyzed the data. J.L. collected serum from healthy individuals and the patient. J.H. performed the drug granules toxicity test. X.L. and C.Z produced the drug granules. X.-F.L., Q.C., H.Z. and L.L. helped specific experiments and data analysis. S.Z. wrote the original draft of the manuscript. Y.-Q.D., S.G. and C.Z. helped revise the manuscript. All authors read and approved the contents of the manuscript.

ADDITIONAL INFORMATION

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Competing interests: C.-F.Q. and H.Y. have filed a patent related to the antiviral activity of 25HC. X.L. is employee of Porton Biologics Ltd. C.Z. is employee of CoSci Me-Tech Co., Ltd.

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REFERENCES

1. Petersen, E. et al. *Lancet Infect. Dis.* https://doi.org/10.1016/S1473-3099(20)30484-9 (2020).
2. Li, C. et al. *Immunity* **46**, 446–456 (2017).
3. Liu, S. Y. et al. *Immunity* **38**, 92–105 (2013).
4. Lund, E. G. et al. *J. Biol. Chem.* **273**, 34316–34327 (1998).
5. Adams, C. M. et al. *J. Biol. Chem.* **279**, 52772–52780 (2004).
6. Shen, B. et al. *Cell* **182**, 59–72 e15 (2020).
7. Wu, D. et al. *Natl Sci. Rev.* **7**, 1157–1168 (2020).
8. Sun, S. H. et al. *Cell Host Microbe* **28**, 124–133 e4 (2020).
9. Nie, J. et al. *Emerg. Microbes Infect.* **9**, 680–686 (2020).
10. Gu, H. et al. *Science* https://doi.org/10.1126/science.abc4730 (2020).
11. Anggakusuma et al. *Hepatology* **62**, 702–714 (2015).
12. Wang, M. et al. *Cell Res.* **30**, 269–271 (2020).
13. Liu, J. et al. *Cell Discov.* **6**, 16 (2020).