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ORIGINAL ARTICLE

Establishment and clinical application of SARS-CoV-2 catch column

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Abstract

Background A certain number of patients with coronavirus disease 2019 (COVID-19), particularly those who test positive for SARS-CoV-2 in the serum, are hospitalized. Further, some even die. We examined the effect of blood adsorption therapy using columns that can eliminate SARS-CoV-2 on the improvement of the prognosis of severe COVID-19 patients.

Methods This study enrolled seven patients receiving mechanical ventilation. The patients received viral adsorption therapy using SARS-catch column for 3 days. The SARS-catch column was developed by immobilizing a specific peptide, designed based on the sequence of human angiotensin-converting enzyme 2 (hACE2), to an endotoxin adsorption column (PMX). In total, eight types of SARS-CoV-2-catch (SCC) candidate peptides were developed. Then, a clinical study on the effects of blood adsorption therapy using the SARS-catch column in patients with severe COVID-19 was performed, and the data in the present study were compared with historical data of severe COVID-19 patients.

Results Among all SCC candidate peptides, SCC-4N had the best adsorption activity against SARS-CoV-2. The SARS-catch column using SCC-4N removed 65% more SARS-CoV-2 than PMX. Compared with historical data, the weaning time from mechanical ventilation was faster in the present study. In addition, the rate of negative blood viral load in the present study was higher than that in the historical data.

Conclusion The timely treatment with virus adsorption therapy may eliminate serum SARS-CoV-2 and improve the prognosis of patients with severe COVID-19. However, large-scale studies must be performed in the future to further assess the finding of this study (jRCTs052200134).

Keywords COVID-19 · Viral adsorption therapy · Clinical study

Introduction

Coronavirus disease 2019 (COVID-19) has spread worldwide and caused a pandemic. Despite the development of vaccines and medications, some patients with COVID-19 still develop acute respiratory distress syndrome and other complications. Cytokine storm and excessive inflammatory response play an important role in the pathogenesis of COVID-19 (IDSA Guidelines on the Treatment and Management of Patients with COVID-19; https://www.idsociety.org/practice-guideline/covid-19-guideline-treatment-and-management/, last updated June 29, 2022). A high serum SARS-CoV-2 viral load is associated with a more severe respiratory disease and inflammation(1). Moreover, there is a correlation between serum SARS-CoV-2 levels and adverse outcomes and mortality(2–5). A meta-analysis showed that SARS-CoV-2 RNAemia was associated with COVID-19 severity and unfavorable clinical outcomes(6).

SARS-CoV-2 binds to host cells via its spike glycoprotein, and the receptor binding domain of the S1 subunit directly binds to the peptidase domain (PD) of human angiotensin-converting enzyme 2 (hACE2). Recent mendelian randomization analyses have focused on hACE2-targeting drugs, which can be used for the early management of COVID-19(7). Human neutralizing antibodies for SARS-CoV-2 target the receptor binding domain(8). Similarly,

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soluble hACE2 can neutralize SARS-CoV-2 and block the systemic spread of the virus to other cells(9). Non-hospitalized patients with COVID-19 were successfully treated with combined casirivimab and imdevimab (10). However, the therapeutic strategies for severe COVID-19 remain limited.

Direct hemoperfusion therapy with the polymyxin B-immobilized polystyrene column (endotoxin adsorption column) (PMX-DHP) has been used in patients with severe COVID-19. It can adsorb cytokines and inflammatory cells(11). A recent study showed that patients with COVID-19 can tolerate Seraph ® 100(12). This hemoperfusion device could reduce pathogens, including several viruses. However, these columns could not specifically adsorb SARS-CoV-2. The current study developed a SARS-CoV-2-adsorbing column (SARS-catch column) by immobilizing a specific peptide, designed based on the amino acid sequence of the PD of hACE2 (13), to the PMX column instead of polymyxin B. Hemoperfusion using the SARS-catch column is expected to adsorb SARS-CoV-2, cytokines, and inflammatory cells observed in PMX-DHP therapy (11). Then, we conducted a clinical study on blood adsorption therapy for patients with severe COVID-19 using a SARS- catch column (jRCTs052200134; Registration on March 15, 2021).

Methods

Pseudovirus adsorption assay

To examine the adsorptive capacity of peptide candidates, peptide-immobilized resin or *N*-chloroacetylated Toray-fiber used in the PMX column was incubated for 1 h at 4 °C with replication-deficient vesicular stomatitis virus (VSV)-based pseudovirus (1×10^5 plaque-forming units), unbearing (Luc-pv) or bearing SARS-2-S (ancestral Wuhan type) (SARS2-pv) whose glycoprotein gene was replaced with the luciferase gene (14, 15). To examine the effect of sterilization on virus adsorption capacity, the peptide-immobilized Toray-fiber, unsterilized or sterilized by gamma ray or high pressure in saline or phosphate-buffered saline, were tested. To assess the adsorption capacity of peptide-immobilized column (SARS-catch column), 200 mL of SARS2-pv or Luc-pv (3×10^5 PFU) was circulated through a PMX column or SARS-catch column for 1 h using a peristaltic pump MasterFlex (Yamato Scientific Co., Ltd.).

VeroE6/TMPRSS2 cells were seeded with a concentration at 1×10^5 cells/ml in a volume of 100 µl on 96-well plates and were incubated in DMEM supplemented with 2% (v/v) heat-inactivated FBS and without G418 at 37 °C and 5% CO_2 for 24 h. After incubation with peptide-immobilized resin, fiber or column, 100 µl of mixture of Luc-pv or SARS-pv solution was added to VeroE6/TMPRSS2 cells, and then incubated at 37 °C and 5% CO_2 for more 24 h. After incubation, washing each well with PBS, each of the cells was completely lysed with 50 µl of Cell Culture Lysis Reagent (Promega), and then 5 µl of lysed solution was transferred to 96 well white microplates (Berthold Technologies, Germany). Finally, the relative light units (RLUs) 400 ms after 20 µl of Luciferase Assay Substrate Solution (Promega) added were measured using a FilterMax F3 Multi-Mode Microplate Readers (Molecular Devices) and analyzed with SoftMax Pro 6.2.1(Molecular Devices).

Affinity assay and enzymatic digestion

Lyophilized peptide biotinylated on cysteine (Cys) was dissolved to 0.2 mg/mL in DMSO and diluted 100-fold into 0.1% BSA, 0.02% Tween-20, and 1 × PBS (kinetic buffer) for immobilization onto streptavidin (SA) biosensors (ForteBio, Fremont, CA). Bilayer interferometry (BLI) assays were performed using the BLItz system (ForteBio, Fremont, CA). Biotinylated peptide was immobilized onto the SA sensors for 120 s. Sensors were then dipped into kinetic buffer for 150 s, protein solutions (prepared in kinetic buffer at concentrations as indicated) for 120 s, and, finally, kinetic buffer for 120 s. Measurements were performed at room temperature. Data were analyzed using the BLItz Pro software with global kinetic fit of all sensorgram curves.

Lyophilized peptides biotinylated on Cys were treated with thermolysin (15 µg/mL) in 50 mM of Tris·HCl pH 8 buffer with 0.5 mM $CaCl_2$ (peptide concentration: 3 mg/mL) at 50 °C for over 240 min. At different time points, aliquots were taken from the reaction mixture and analyzed by RP-HPLC to evaluate the enzymatic stability of peptides.

Clinical procedure

A clinical study was performed to evaluate the efficacy and safety of the novel virus adsorption therapy in patients with severe COVID-19 (jRCTs052200134). Patients with severe COVID-19 who tested positive in the nucleic acid test of blood SARS-CoV-2 and who required an artificial ventilator or extracorporeal membrane oxygen (ECMO) were enrolled in the current study. Patients received blood adsorption therapy using an SARS-catch column at a blood flow rate of 8 to 12 mL/min for 6–8 h on three consecutive days. Nafamostat mesylate (40 mg/h) and heparin (500–2000 U/h) were used in anticoagulant therapy. The primary outcome was withdrawal from ventilator or ECMO, and the secondary outcomes were the ratio of patients who then tested negative for the virus in their blood and improvement in respiratory function (PaO_2/FiO_2 ratio). Serum samples were examined using the SARS-CoV-2 Direct Detection RT-qPCR Kit (Takara Bio, Shiga, Japan) with Roche LightCycler 96 for detecting SARS-CoV-2.

The respiratory function (PaO_2/FiO_2 ratio) and the extent of change from days 1 to 7 and 14 were summarized in patients with a ventilator in the efficacy analysis population. The PaO_2/FiO_2 ratios on days 1 and 7 or days 1 and 14 were compared using the paired *t* test. The measured blood viral level and the changes from days 1 to 4, 7, and 14 in patients in the efficacy analysis population were summarized, and the blood viral levels on days 1 and 4, days 1 and 7, or days 1 and 14 were compared using the paired *t*-tests.

The data in this study were compared with the historical cohort data of patients admitted to the intensive care units due to COVID-19. Data were presented as mean and standard deviation for continuous variables and frequency and proportion for categorical variables. The withdrawal rate and the median day required for withdrawal were estimated using the Kaplan–Meier (KM) method. The changes in PaO_2/FiO_2 ratio from day 1 to days 7 and 14 were evaluated using the paired *t*-test. Further, the changes in blood viral level from day 1 to days 4, 7, and 14 were compared using the paired *t*-tests. To compare the rate of withdrawal from ventilator use, the KM method and the log-rank test were used. Meanwhile, the start time point was set to the day of ventilator care initiation. The difference in PaO_2/FiO_2 ratio at days 7 and 14 were compared using the *t*-test. The qualitative test of serum SARS-CoV-2 was used as the outcome. The negative rates on days 4, 7 and 14 were compared using the Fisher's exact test. Missing PaO_2/FiO_2 ratio values and blood viral levels were imputed with the paired *t*-test. Missing qualitative blood viral tests were also imputed based on previous results.

Results

Design and synthesis of SARS-CoV-2-catch peptide candidate

We designed eight types of SARS-CoV-2-catch (SCC) candidate peptides based on hACE2 (21–43), where SARS-CoV-2 spike glycoprotein binds(13), and miniPEG-linked Cys was introduced at the N- or C-terminal for attachment to a solid support (Fig. 1a, Supplementary file 1). Peptides containing Cys within the N-terminal and C-terminal regions were referred as N and C types, respectively. Among the peptides designed in this study, SCC-1 N/1C contained hACE2(21–43). In terms of the structure of SCC-2 N/2C, considering that hACE2(21–43) interacts with SARS-CoV-2 by possessing the α-helix structure, we introduced three 2-aminoisobutyric acid (Aib) residues, which increase the α-helicity of the peptide, on the opposite side from the spike

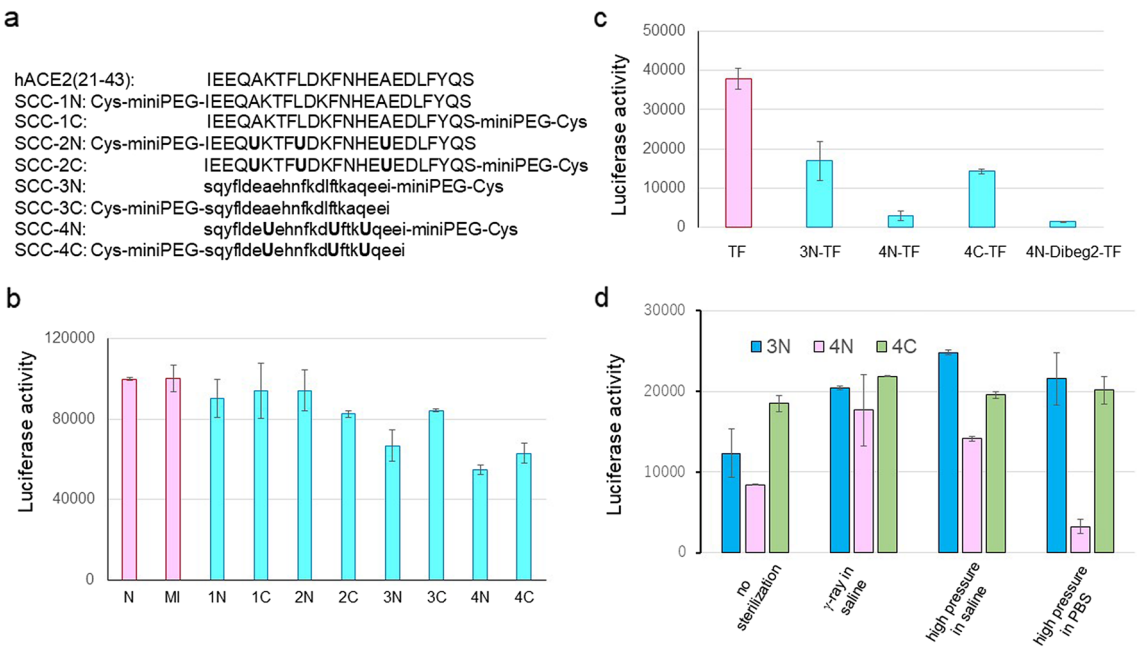

Fig. 1 Eight types of candidate peptides were designed based on hACE2(21–43). SCC-2/4 contains Aib (U), and SCC-3/4 is a retro-inverso peptide, where lowercase letters indicate D-amino acids. Peptides containing Cys within the N-terminal region are referred to as N-type and peptides containing Cys within the C-terminal region as C-type. This N/C nomenclature is based on the native sequence; thus, it does not match the chemical structures in case of retro-inverso peptides (SCC-3/4) **a**. The viral adsorption activities of candidate peptides were assessed using a pseudovirus adsorption assay. Amino-resin (N) and maleimide-resin (MI) were used as negative controls **b**. Viral adsorption activities of three types of candidate peptides immobilized on the chloroacetylated Toray fiber were used as a negative control **c**. The deterioration effect of sterilization was evaluated with a pseudovirus adsorption assay **d**

protein binding side to prevent disrupting the peptide–protein interaction. Moreover, we adopted the retro-inverso strategy (16) to identify tolerance against enzymes to design SCC-3/4 based on SCC-1/2.

Adsorption capacity of the SCC candidate peptides

To investigate the adsorption activity of the SCC candidate peptides, SCC candidate peptide-immobilized purolite resins were examined using the pseudovirus adsorption assay, which is the initial screening, and amino- and maleimide-purolite resins were used as negative controls. The luciferase activities of the infected cells were reduced in SCC-3N-, SCC-4C-, and SCC-4N-immobilized resin-treated SARS-pv solution (Fig. 1b). Based on these positive results, SCC-3N-, SCC-4C-, or SCC-4N-immobilized Toray fiber were then tested using the pseudovirus adsorption assay because Toray fiber will be adopted as a solid support in clinical studies. As shown in Fig. 1c, SCC-4N on the Toray fiber had the best adsorption activity. To examine the effect of the change in miniPEG moiety on the adsorption activity, SCC-4N-Dibeg2 was also examined. Eventually, over 95% of psuedovirus adsorption was confirmed in SCC-4N-Dibeg2-immobilized Toray fibers. Since sterilization is necessary for the clinical application of the SARS-catch column, we also evaluated whether the virus adsorption capacity could not be weakened by sterilization. Results showed that the virus adsorption capacity was retained if the peptide-immobilized fibers were placed in PBS solution and sterilized under high pressure (Fig. 1d)

To confirm this finding, the physicochemical affinity of SCC-4N was compared with that of SCC-1N, which was used as the native control. As shown in Fig. 2a, in the biolayer interferometry (BLI) using spike protein as an analyte, a Kd value of 2.4 μM was observed in SCC-4N. By contrast, the Kd value of SCC-1N could not be determined because no significant binding was detected. In addition, enzymatic stabilities of peptides were evaluated using thermolysin, which is a robust enzyme for peptide mapping. As shown in Fig. 2b, SCC-1N was decomposed within 10 min. However, SCC-4N comprises non-native D amino acids. Thus, it was not significantly affected under the same condition over 240 min.

Based on the adsorption activity of SCC, we adopted a blood adsorption column containing SCC-4N-Dibeg2, which is the most effective peptide. The SARS-catch column was prepared by immobilizing SCC-4N-Dibeg2 via the thiol group of Cys to the Toray fiber, and was assembled in the same way as the PMX column. Finally, 200 mL of SARS2pv (1.5×10^4 PFU/mL) was circulated via the SARS-catch or PMX column using a peristaltic pump for 1 h at room temperature. The SARS-CoV-2 adsorption capability of the SARS-catch column was better than that of the PMX column (65% reduction in luciferase activity, Fig. 3a).

Efficacy and safety of clinical study

Since the stability and safety of the SARS-catch column was confirmed (Supplementary file 2), the safety and efficacy of blood adsorption therapy were investigated using the SARS-catch column in patients with severe COVID-19. Seven patients who are on mechanical ventilator were enrolled in the current study (Table 1), and they received viral adsorption therapy using the SARS-catch columns for 3 days (6 to 8 h per day). In this study, four of seven patients were weaned from the ventilator until day 14 (which is the day when adsorption therapy was initiated, designated as day 1.), with a withdrawal rate of 0.571 on day 14 and a median time to withdrawal of 5.0 days. The respiratory function of patients (PaO_2/FiO_2 ratio) did not improve based on the mean measurements values in day 7 (mean change: 1.93 ± 97.0, $p = 0.96$) and day 14 (mean change: 12.2 ± 78.6, $p = 0.70$) compared with day 1, probably because of missing values, which could be high if observed, due to early improvement and weaning from ventilator. The mean blood SARS-CoV-2 RNA levels were significantly lower in all visits (day 4 -0.144 ± 0.127, $p = 0.024$; day 7 -0.143 ± 0.133, $p = 0.030$; day 14 -0.151 ± 0.127, $p = 0.020$), and SARS-CoV-2 RNA was not detected on day 14. Of five patients whose blood SARS-CoV-2 RNA levels could be evaluated

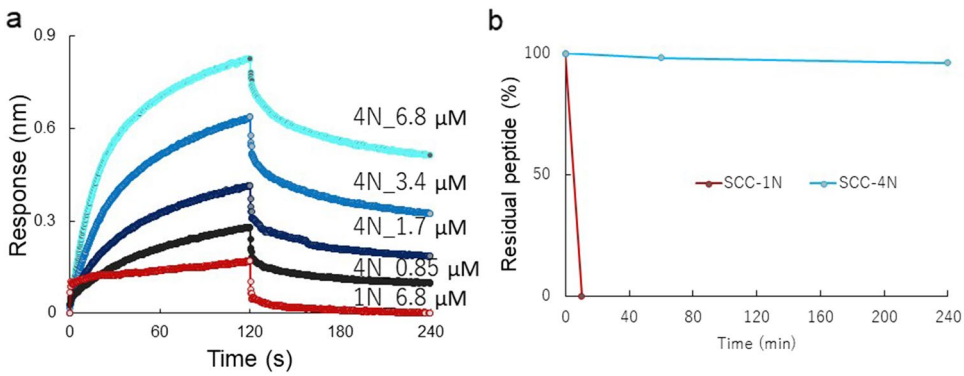

Fig. 2 Comparison between SCC-1N and SCC-4N based on BLI traces (**a**) and enzymatic stability (**b**)

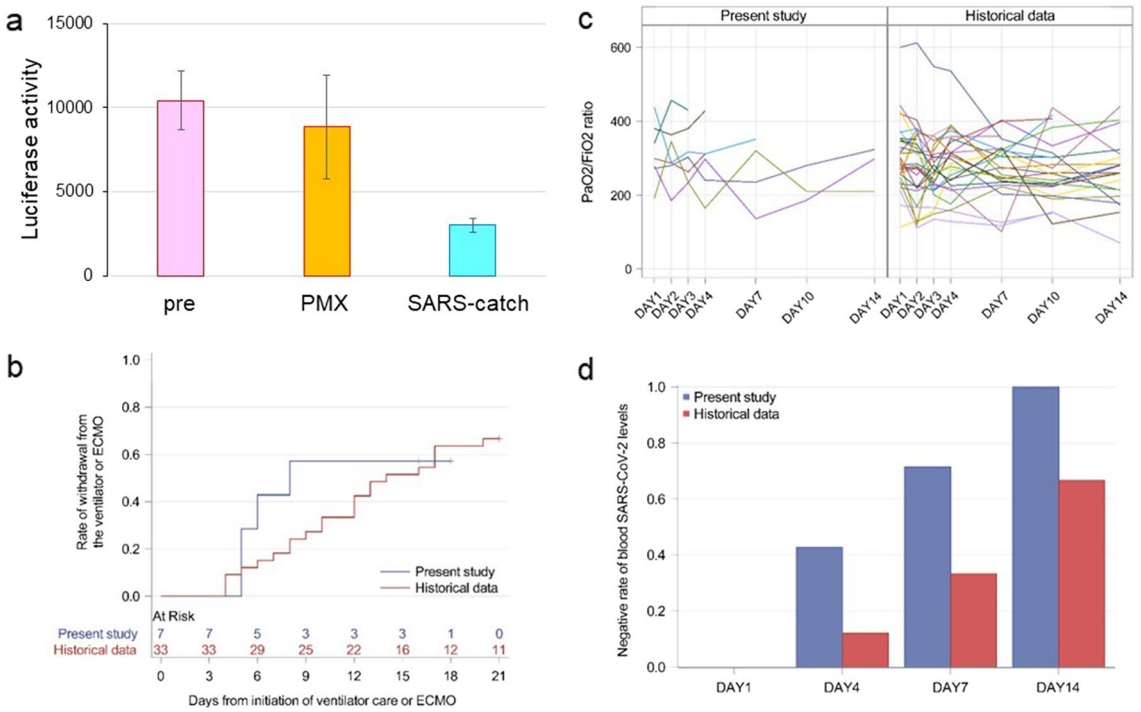

Fig. 3 Efficacy of SARS-catch column. Comparison of SARS-CoV-2 adsorption ability between the PMX column and SARS-catch column before (pre) and after pseudovirus perfusion (**a**). Comparison of withdrawal time from ventilator or ECMO (**b**), longitudinal data of respiratory function tests (PaO_2/FiO_2 ratio) of each patient (**c**) and rate of negativity to blood SARS-CoV-2 (**d**) between the current and historical data. In this study, the maximum observation period was 18 days. To compare respiratory function **c** and blood SARS-CoV-2 levels (**d**), the historical data at day 1 were shifted and analyzed using the median number of days from ventilator placement to the start of adsorption therapy in the current study

Table 1 Baseline characteristics in current study and historical cohort data

	Current study	Historical cohort
Number	7	33
Age (mean ± SE)	56.9 ± 17.5	70.6 ± 10.1
Gender (male)	7 (100.0%)	21 (63.6%)
Body mass index (BMI)	25.5 ± 5.2	24.2 ± 3.7
Comorbidity		
Hypertension	4 (57.1%)	17 (51.5%)
Diabetes	2 (28.6%)	13 (39.4%)
Cardiovascular disease	0 (0.0%)	3 (9.1%)
Respiratory disease	0 (0.0%)	8 (24.2%)
Renal disorder	2 (28.6%)	8 (24.2%)
(dialysis)	2 (28.6%)	4 (12.1%)
Dyslipidemia	3 (42.9%)	16 (48.5%)
Smoking		
Present	0 (0.0%)	2 (6.1%)
Past	3 (42.9%)	16 (48.5%)
Non-smoking	4 (57.1%)	13 (39.4%)
Unknown	0 (0.0%)	2 (6.1%)
Time from onset to ventilator or ECMO placement (day ± SE)	6.1 ± 4.6	8.4 ± 3.3

after the first day of adsorption therapy, one patient tested negative after adsorption therapy, but returned positive on the next day.

Since the SARS-catch column is a modified endotoxin adsorption column, it is expected to have cytokine adsorption capacity. Therefore, we examined the inflammatory response before and after the viral adsorption therapy using SARS-catch column (day 1 and day 4) in the patients in the current study (Fig. 4), and found that CRP (7.91 ± 10.0 on day 1 and 3.68 ± 3.38 mg/dL on day 4, $p = 0.15$; Fig. 4a), Interleukin-4 (IL-4) (17.19 ± 22.60 and 13.48 ± 16.57 pg/mL, $p = 0.09$; Fig. 4b), and Interferon-γ (INF-γ (0.14 ± 0.07 and 0.12 ± 0.04 IU/mL, $p = 0.47$; Fig. 4c) showed a decreasing trend, but increased in patients with bacterial pneumonia (patient 5) and systemic mycosis (patient 6), and no significant difference was observed. D-dimer was also measured before and after viral adsorption therapy, since vascular endothelial damage has been reported to complicate COVID-19 infection. There was no significant difference in D-dimer levels (3.39 ± 1.70 and 3.68 ± 2.86 μg/mL, $p = 0.37$; Fig. 4d).

We compared the current study data with the historical cohort data of patients admitted to the intensive care units due to COVID-19 at Osaka University Hospital before the

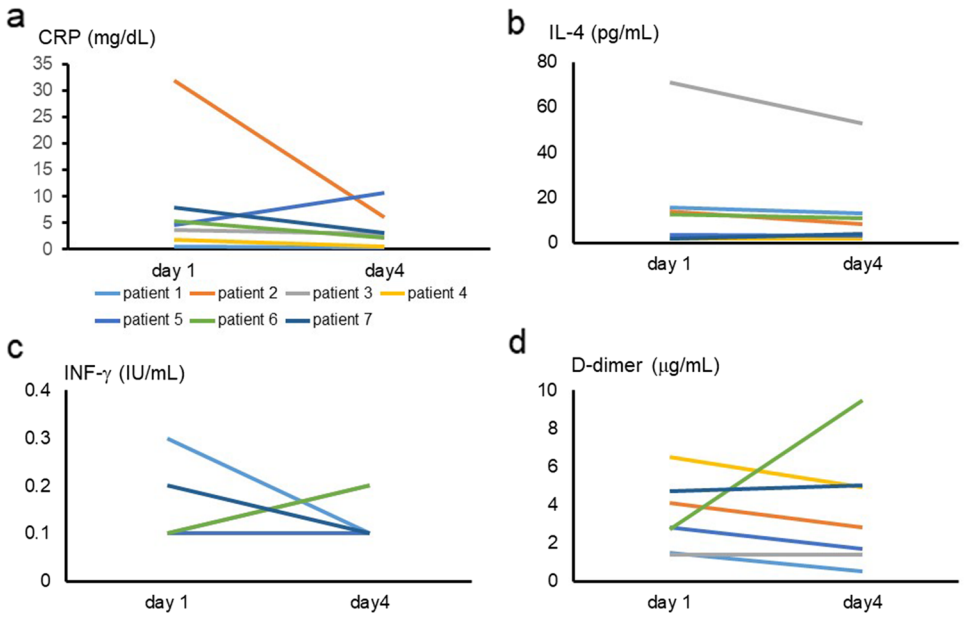

Fig. 4 The change of CRP **a**, IL-4 **b**, INF-γ **c** and D-dimer **d** were examined before (day 1) and after (day 4) viral adsorption therapy in 7 patients.

Table 2 Combined therapy in current study and historical cohort data

	Current study	Historical cohort
Number	7	33
RAS inhibitor	1 (14.7%)	1 (3.0%)
Steroid therapy	7 (100.0%)	33 (100%)
Anti-viral drug	5 (71.4%)	25 (75.8%)
Ivermectin	5 (71.4%)	0 (0%)
Remdesivir	1 (14.3%)	17 (51.5%)
Favipiravir	0 (0.0%)	18 (54.5%)
Tocilizumab	0 (0.0%)	2 (6.1%)
Abatacept	0 (0.0%)	1 (3.0%)
Heparin therapy	7 (100.0%)	33 (100%)
Prone therapy	7 (100.0%)	33 (100%)

RAS renin–angiotensin system

initiation of the current study. Patients enrolled in the current study (7 patients) tended to be younger ($p=0.086$), had a higher BMI ($p=0.27$), and were less likely to have diabetes ($p=0.691$), cardiovascular disease ($p=1.000$) and respiratory disease ($p=0.309$) than those enrolled in the historical cohort (33 patients), although there were no significant differences (Table 1). All of the severe COVID-19 patients in the current study and the historical cohort study received steroid therapy, heparin therapy, and prone therapy. There were no significant differences in the proportion receiving antiviral therapy, but the antiviral medications received tended to vary depending on when the patient was receiving treatment (Table 2). The median withdrawal time from the ventilator was 5 days in the current study. Meanwhile, it was 14 days based on the historical data. Therefore, the time to weaning was faster in the current study (Fig. 3b). The rate of mechanical ventilator withdrawal was 0.429 based the current study data and 0.152 according to the historical data on day 7. This value was about three times higher than that in the current study. However, the values did not significantly differ on day 14 (0.571 and 0.485, respectively). The p-value of the log-rank test was 0.777. Therefore, there was no statistically significant difference in terms of the rate of mechanical ventilator withdrawal during the whole observation period. There were minimal differences in terms of changes in PaO_2/FiO_2 ratio from day 1 to day 7 (1.93 ± 97.0 vs -25.6 ± 72.3, $p=0.395$) and day 14 (12.2 ± 78.6 vs -22.4 ± 105, $p=0.419$) between the current study data and historical data (Fig. 3c). The proportion of patients who tested negative for blood SARS-CoV-2 increased from day 4 to day 7 or 14 based on the current study data and the historical data. However, the results were not significant owing to the small sample (day 4: 0.429 vs 0.121, $p=0.088$; day 7: 0.714 vs 0.333, $p=0.094$; and day 14: 1.000 vs 0.667, $p=0.16$) (Fig. 3d).

Discussion

In this study, we developed the SARS-catch column by immobilizing peptides, based on the sequence of hACE2, to an endotoxin adsorption column, which was clinically used for severe COVID-19 patients (11), and demonstrated that the SARS-catch column using SCC-4N removed 65% more SARS-CoV-2 than the endotoxin adsorption column. Hence, we examined the effect of blood adsorption therapy using SARS-catch column on the improvement of the prognosis of severe COVID-19 patients. Compared with historical data of severe COVID-19 patients, the weaning time from

mechanical ventilation was faster, and the rate of negative blood viral load was also higher in the present study.

We designed eight types of SCC candidate peptides based on hACE2 and found that SCC-4N on the Toray fiber had the best adsorption activity, suggesting that retro-inverso modification increased the affinity of the peptide against SARS-CoV-2. Generally, structured peptides are not suitable targets of retro-inverso strategy because secondary structures are not retained in retro-inverso peptides(17). Hence, the most effective SCC-4N designed using the retro-inverso strategy for enzyme resistance coincidentally increased the affinity of peptides against the SARS-CoV-2 S protein. We are still investigating why retro-inverso peptides worked well in this study. To reduce the cost of further large-scale synthesis, miniPEG moiety between the peptide and Cys was modified from $(AEEA)_3$ used in the initial screening to two-times-repeated diethylene glycol bis(3-aminopropyl) ether-glutaric acid (i.e., $(Dibeg)_2$), and the modified SCC-4N-Dibeg2 was also effective in the adsorption activity. This is attributed to the fact that $(AEEA)_3$ and $(Dibeg)_2$ have a similar length.

Compared with historical data of severe COVID-19 patients, the weaning time from mechanical ventilation was faster, and the rate of negative blood viral load was also higher in the present study. Although a high serum SARS-CoV-2 viral load is associated with a more severe respiratory disease and inflammation(1), it is not clear why reducing SARS-CoV-2 virus in the blood improves respiratory function. When we examine the course of severe COVID-19 patients admitted to our hospital with positive blood SARS-CoV-2 virus, we found that after the blood virus first disappeared, the virus in the pharynx became negative, and then the virus in the nasal cavity became negative. Although changes in viral levels in the upper and lower respiratory tracts have not been examined in the current study, it is possible that the decrease in blood virus levels may have hastened the decrease in viral levels in the upper and lower respiratory tracts and improved respiratory function. The respiratory function of patients (PaO_2/FiO_2 ratio) was improved neither in this study nor in the historical cohort. This is due to the fact that respiratory function data (PaO_2/FiO_2 ratio) were only used under ventilatory management, which excluded data after patients were weaned from the ventilator. One of the enrolled patients with hemodialysis developed bacterial pneumonia on day 5, which delayed weaning from the ventilator, but the Efficacy and Safety Evaluation Committee ruled out a causal relationship with virus adsorption therapy. One patient had a delayed weaning from the ventilator because of disquiet due to comorbid mental illness. No adverse events were observed, although treatment was discontinued prematurely after initiation due to the presence of clots in the column. Since patients with severe COVID-19 are often complicated by vascular endothelial damage, heparin therapy is used in our hospital. No worsening of D-dimer was observed after the viral adsorption therapy in the patients in the current study.

In this study, the mean blood SARS-CoV-2 RNA levels were significantly lower in all visits than those on the day 1, and SARS-CoV-2 RNA was not detected on day 14. Of five patients whose blood SARS-CoV-2 RNA levels could be evaluated after the first day of adsorption therapy, four tested positive, and one patient who tested negative also had a positive blood RNA level on the next day. Hence, viral adsorption therapy for three consecutive days can effectively eliminate viruses from the blood because intracellular viruses cannot be removed via column adsorption therapy. Most of the patients enrolled in the current study received 8 h of viral adsorption therapy for 3 consecutive days at a blood flow rate of 12 mL/min (5.7 L of viral adsorption per day). One of the 5 patients became negative for virus after adsorption therapy but was positive for virus the following day. This may have been due to the release of intracellular virus into the blood. However, the proportion of patients who became negative for virus after 3 consecutive days of the viral adsorption therapy was higher than in the historical cohort. Originally, it was anticipated that a 24-h continuous viral adsorption therapy for 3 consecutive days would have a greater therapeutic effect, but because medical devices that have a cumulative contact time with blood and other substances exceeding 24 h require more sophisticated biological safety testing, the current study was therefore conducted using viral adsorption therapy for 3 consecutive days, 8 h per day.

In this study, four of seven patients were weaned from mechanical ventilator during the observational period. In addition, an exploratory comparison between the current study data and historical data showed a trend toward earlier negative blood viral load in patients treated with viral adsorption therapy. However, the historical data were limited because multiple strains of SARS-CoV-2 mutated during the course of the pandemic, and the severity of infection varied with each mutant strain. The mutant strain of enrolled patients in the current study and the historical cohort study was not examined in our hospital. Further investigation of column adsorption capacity for SARS-CoV-2 virus mutant strains is needed. No serious adverse events were observed. However, treatment was discontinued prematurely after initiation due to the presence of clots in the column in the first adsorption therapy of this study due to lack of anticoagulants. All of our patients with severe COVID-19 were treated with heparin, and in the first case, the anticoagulant dose was reduced to avoid bleeding risk, which contributed to the clotting in the column, but thereafter, the anticoagulant was adjusted and no clotting in the column was observed.

The current study showed that the timely administration of virus adsorption therapy may eliminate serum SARS-CoV-2 and improve respiratory function among

patients with severe COVID-19. However, future large-scale studies must be conducted to further assess this finding. Patients enrolled in the current study tended to have a lower risk of severe COVID-19 infection than those enrolled in the historical cohort, although the difference was not significant. However, it is conceivable that these differences could have affected the duration of withdrawal from the ventilator. In addition, patients enrolled in the historical cohort were not vaccinated, because the historical cohort enrolled patients who became severely ill due to COVID-19 before the ethics application for the current study was approved. However, two of the seven patients enrolled in this study had been vaccinated. The possibility that vaccination status affected the duration of withdrawal from the ventilator cannot be ruled out. Direct hemoperfusion therapy using endotoxin adsorption column was found to adsorb cytokines and inflammatory cells among patients with COVID-19 patients(11). Two of the seven patients had bacterial pneumonia or systemic mycosis with worsening CRP and cytokines, while the inflammatory response tended to improve in the other patients. Removal of SARS-CoV-2 virus from the blood may improve systemic inflammatory conditions. However, whether the removal of SARS-CoV-2 with the SARS-catch column can further improve the prognosis of COVID-19 patients remains to be elucidated.

The current study had several limitations. That is, only seven patients were enrolled in this study. Although patients who are critically ill patients and are on mechanical ventilation were included, virus adsorption therapy using the SARS-catch column may be more effective at the earlier phase of moderate to severe COVID-19 with positive serum SARS-CoV-2. Considering that both SARS and SARS-CoV-2 viruses can enter cells via hACE2, the SARS-catch column may also be useful during another coronavirus pandemic targeting hACE2. Furthermore, similar to SARS-CoV-2, the virus adsorption strategy using such columns may be useful for viral diseases (such as Ebola hemorrhagic fever and severe fever with thrombocytopenia syndrome) in which viremia is frequent and blood viral levels are associated with disease severity.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest to disclose.

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