

First Aid Disinfection by Gargling Electrolyzed Saline for COVID-19 Infections

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ABSTRACT

Introduction: Electrolyzed saline (ES) is a non-toxic virucidal disinfectant. A treatment strategy for COVID-19 infections, in which initial decontamination was achieved by gargling ES, was retrospectively investigated.

Methodology: Thirty-eight COVID-19 patients were admitted between June 8, 2021, and September 30, 2021. Each patient gargled ES 3 times a day for as long as salivary SARS-CoV-2 antigen (Ag) tests produced positive results. Treatment effects were evaluated based on the salivary Ag level.

Results: Gargling ES solution was used to decontaminate the primary infection site in 33 patients (study group), whereas it was used at 7–10 days after admission in 5 patients (control). Thirty patients were <65 y.o., and 12 were female. Twenty-six exhibited pneumonias on CT. 13 patients were fully vaccinated, which reduced the risk of pneumonia (OR: 4.67, 95%CI: 1.08-20.22). Gargling ES significantly reduced the duration of the Ag-positive period and salivary Ag levels. An Ag-positive rate of 50% was achieved within 7 and 17 days in the study and control groups, respectively ($p=0.0013$). All pneumonia shadows faded away as the salivary Ag level reduced. All of the patients were released from quarantine within 20 days.

Conclusion: ES gargling rapidly reduced Ag levels and suppressed the progression of COVID-19.

Keywords

COVID-19, Electrolyzed saline, First aid disinfection.

Introduction

Electrolyzed saline (ES) is a non-toxic, all-purpose disinfectant, which kills viruses; bacteria, except spore-forming bacteria; and fungi [1,2]. The US Food and Drug Administration (FDA) approved ES as a high-level disinfectant in October 2002 [3]. We have used ES as a disinfectant and sterilizer against the bio contamination of residential spaces, medical equipment, skin, mucosae, and the closed pleural/abdominal cavity since 1993 [1,4]. We routinely use ES gargling for preoperative oral hygiene [4], and in a preliminary study, we reported that gargling ES promptly reduced SARS-CoV-2 antigen (Ag) levels and ameliorated COVID-19 infections [5]. In this study, the effects of our treatment strategy,

in which gargling ES was employed as an initial treatment, were retrospectively investigated.

Methodology

The institutional review board of our hospital granted ethical approval for this study (IRB approval #03-05, SMH, May 25, 2021). Informed consent was obtained from each patient.

Patients from the regional COVID-19 monitoring center with no, mild, or moderate symptoms who were admitted to our hospital, which has been designated an official hospital for COVID-19 treatment, between June 8, 2021, and September 30, 2021, were included in this study. Ten beds were prepared for COVID-19 treatment. At the start of the study period, the vaccination of individuals aged >65 y.o. had just finished, and the vaccination

of younger individuals was ongoing during the study period. The patients' characteristics were compared with those of patients with conventional community-acquired pneumonia (Conv Pneumonia) who were admitted between January 1, 2018, and December 31, 2019; i.e., before the COVID-19 era [6]. The patients that underwent ES gargling on admission were defined as the study group, and those who refused to undergo ES gargling on admission but underwent ES gargling 7~10 days later after the confirmation of persistent salivary Ag positivity were defined as the control group. The associations between the patients' characteristics and clinical features and pneumonia being detected on computed tomography (CT) were evaluated. The effects of treatment were evaluated based on the salivary Ag level. The Ag-positive period was assumed to start on the day before an Ag level of ≥ 4.0 pg/ml was confirmed. The discharge criteria included the relief of symptoms, the confirmation of fading pneumonia shadows on CT, and the reduction of the salivary Ag level to < 20 pg/ml. The endpoints were salivary Ag levels, and the duration of the patients' hospital stays.

Treatment strategy for COVID-19 infections

The treatment of viral pneumonia generally followed the Japanese Guidelines for Acute Respiratory Distress Syndrome 2016 [7] with some modifications.

Initial decontamination of the primary infection site ES gargling and the nasal injection of ES solution were used to decontaminate the primary infection site. Each patient gargled a glass of ES and then received bilateral 2-ml nasal injections of ES with a 5-ml syringe on the day of admission (day 1). This procedure aimed to disinfect any superficial virus particles and prevent droplet-borne transmission. Each patient then gargled half a glass of ES with/without nasal injections 3 times a day for as long as salivary Ag tests produced positive results. This procedure aimed to disinfect any parasitic virus particles present in host cells.

Anti-viral drug therapy

Anti-viral drug treatment was performed according to the National Institutes of Health guidelines [8]. Remdesivir (Veklury[®]; Gilead Sciences, USA) was administered for 5 days when pneumonia was observed on the initial CT scan [9]. An antibody cocktail containing casirivimab and imdevimab (Ronapreve, Chugai Pharmaceutical Co., Ltd., Tokyo) was administered to patients who had flu symptoms, but did not exhibit pneumonia on CT. Anti-viral drugs were not administered to patients who were free of symptoms and did not exhibit pneumonia. Anti-viral drugs were delivered from the Japanese government 2 days after placing orders.

Suppression of pulmonary interstitial edema

Blowing up a rubber balloon or glove was used to induce a positive end-expiratory pressure (PEEP) load and prevent permeability edema. This method was employed as an alternative to PEEP by mechanical ventilation [10].

Supplemental oxygenation

If a patient complained of dyspnea, minimal oxygen was given to maintain their percentage oxygen saturation (%SpO₂) between 90% and 95%, while reducing the production of oxygen radicals [10-12].

Online monitoring of disease changes

The patients' symptoms (fever, general fatigue, appetite loss, naso-oral sensory defects, and dyspnea), %SpO₂ values, blood test results, and salivary or nasal swab SARS-CoV-2 Ag levels (according to the Lumipulse SARS-CoV-2 Ag test; Lumipulse G1200, FUJIREBIO, Tokyo) and whether they exhibited pneumonia shadows on CT images were monitored. A positive Ag test result was defined as an Ag level of ≥ 4.0 pg/ml, and the maximum detectable Ag level was assumed to be 5000 pg/ml. Symptoms and %SpO₂ values were monitored every day; chest CT scans were carried out on admission and on days 2, 4, 7, and as needed before discharge; and salivary Ag tests were performed on admission (before ES gargling) and then every 3 or 4 days thereafter (in the early morning). A nasal swab Ag test was performed appropriately just before discharge.

Custom-made ES preparation

ES quickly loses its disinfection activity. A custom-made ES preparation was used in this study [1]. Aqueous ES solution was produced from a 0.1% salt/tap water mixture using a water electrolysis generator (Oxilyzer Medical C-L, Koken Ltd., Tokyo). Electrolyzed acidic water with a pH of < 2.7 was generated at the anode compartment and was collected for immediate use.

Statistical analyses

Categorical variables (the patients' characteristics and clinical features) were evaluated using odds ratios (OR). Treatment effects were evaluated using the Cox proportional hazards model or Kaplan-Meier curves. Reductions in Ag levels are shown in scatter diagrams with regression lines. P-values of < 0.05 according to χ^2 test, log-rank test, or F-test were considered to be significant. Microsoft Excel software (Excel statistics 2020; Ekuseru-Toukei 2020, Social Survey Research Information Co. Ltd., Tokyo) was used for all statistical analyses.

Results

Thirty-eight patients were admitted to our hospital during the 4 months of the 5th pandemic wave in Japan [13].

Comparison of the patients' characteristics with those patients with conventional community-acquired pneumonia (Conv Pneumonia) (Table 1).

Table 1: Characteristics of the patients in the COVID-19 and conventional community-acquired pneumonia groups.

	COVID-19	Conv pneumonia	OR	95%CI
Age: < 65 y/ ≥ 65 y	30/8	92/241	9.8234	4.3437-22.2159
Sex: male/female	26/12	186/147	1.7124	0.8357-3.5088
Charlson score: 0,1/ ≥ 2	36/2	128/205	28.8281	6.8241-121.7841
Current smoker: yes/no	9/29	16/317	6.1487	2.4978-15.1360

Conv-pneumonia: conventional community-acquired pneumonia, Charlson score: Charlson comorbidity score (0 or 1: no or mild comorbidities, ≥ 2 : moderate or severe comorbidities), OR: odds ratio, CI: confidence interval.

Both the COVID-19 and Conv Pneumonia groups included more males than females. A high proportion of the COVID-19 group were aged <65, while the Conv Pneumonia group included more elderly patients [OR: 9.82, 95% confidence interval (95%CI): 4.34-22.22] and patients with comorbidities [a Charlson comorbidity score of >2 (1)] (OR: 6.82, 95%CI: 6.82-121.78). The lower frequencies of elderly patients and patients with comorbidities in the COVID-19 group may have been due to the effects of the Japanese vaccination program. Being a non-smoker was previously identified as a high-risk factor for Conv Pneumonia [6], but being a current smoker did not reduce an individual's risk of being infected with COVID-19 (OR: 6.15, 95%CI: 2.50-15.14). The percentage of current smokers among the COVID-19 patients was the same as that seen among the general population in our region [6].

Table 2: Risk factors for COVID-19 pneumonia.

Risk Factors	Pneumonia (-)	Pneumonia (+)	OR	95%CI
Age: <65 y/≥65 y	6/6	24/2	0.833	0.0133-0.5212
Sex: male/female	6/6	20/6	0.3000	0.0701-1.2835
Current smoker: yes/no	3/9	6/20	1.1111	0.2258-5.4684
Fully vaccinated: yes/no	7/5	6/20	4.6667	1.0772-20.2170
Symptoms: no/yes	3/9	2/24	4.0000	0.5712-28.0111
Ag level: <500 pg/≥500 pg	6/6	7/19	2.7143	0.6526-11.2889

OR: odds ratio, CI: confidence interval.

Risk of pneumonia in the COVID-19 group (Table 2). Twenty-six patients already had pneumonia on admission. Three of them exhibited pneumonia shadows soon after admission. Elderly patients (aged >65 y.o.) were at lower risk of pneumonia (OR: 0.833, 95%CI: 0.0133-0.5212), and vaccination suppressed the progression to pneumonia (OR: 4.667, 95%CI: 1.077-20.217). Sex, comorbidities, smoking status, symptoms, and the salivary Ag level on admission were not found to be risk factors for pneumonia.

The effects of the treatment intervention

Thirty-three patients underwent ES gargling on admission (study group), and the remaining 5 underwent delayed ES gargling (control group). Twenty-five patients in the study group received anti-viral drug treatment for mild or moderate disease with/without pneumonia. Eight patients in the study group were not treated with anti-viral drugs, as they had asymptomatic disease and were free from pneumonia. Four patients in the control group received anti-viral drug treatment for mild or moderate disease with or without pneumonia, and the remaining patient was not treated with anti-viral drugs, as they had asymptomatic disease and were free from pneumonia.

The salivary Ag levels of all 38 patients rapidly reduced (Figure 1). The speed of the reduction in the salivary Ag level after ES gargling was almost the same in both the study (Ag-level=178.2e^{-0.448day}, R²=0.7098) and control (Ag-level=23485e^{-0.465day}, R²=0.8389) groups, although these changes occurred 10 days later

in the latter group. If it is assumed that superficial virus particles were instantly disinfected by the ES solution, it can be speculated based on the above-mentioned regression lines that one third of parasitic virus particles were eradicated every night by daily ES gargling. Twenty-six patients already had pneumonia shadows on early-stage CT images, and all pneumonia shadows faded away with or without residual organized changes (Figure 2). In addition, all of the patients' clinical symptoms improved rapidly.

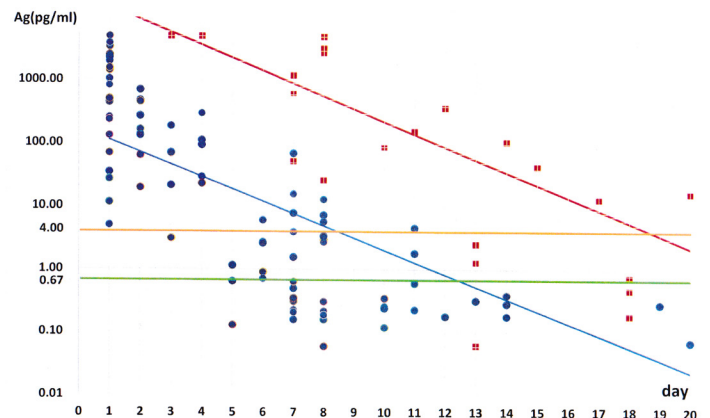


Figure 1: Reduction in SARS-CoV-2 antigen level induced by gargling electrolyzed saline: Initial gargling vs. delayed gargling (logarithmic graph).

Blue dots: initial electrolyzed saline (ES) gargling (study) group, red dots: delayed ES gargling (control) group.

Regression lines (non-parametric): blue: SARS-CoV-2 antigen (Ag) level (pg/ml) = 178.2e^{-0.448day}, R²=0.7098, F=42.2, p<0.001; red: Ag level = 23485e^{-0.465day}, R²=0.8389, F=12.7, p=0.0035.

Above orange line: COVID-19-positive; below green line: COVID-19-negative; vertical axis: salivary Ag level (pg/ml); horizontal axis: time since admission (days); ES: electrolyzed saline.

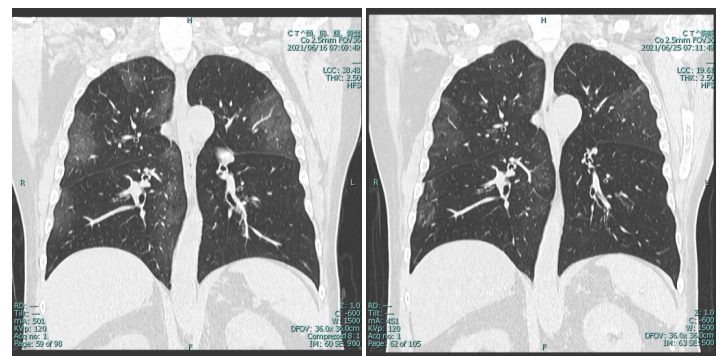


Figure 2: Amelioration of COVID-19 pneumonim.

A 71 y.o. male patient exhibited multiple non-segmental diffuse pneumonia lesions with fine interstitial consolidation on computed tomography (CT) images on day 2 after admission. (Left) CT performed on day 10 after admission showed that the pneumonia shadows had faded after he started gargling electrolyzed saline, and his lungs were free from organized changes. (Right)

According to Kaplan-Meier curve analysis, it took 7 and 17 days to achieve an Ag-positive rate of 50% in the study and control groups, respectively ($p=0.0013$; Figure 3A). In addition, there was a significant difference in the time required to reach a 50% hospitalization rate between the initial (13 days) and delayed (17 days) ES gargling groups ($p=0.0041$). All of the patients were discharged within 20 days without progressing to respiratory failure and returned to their normal lives (Figure 3B). None of them experienced COVID-19 relapses. Initial ES gargling reduced the salivary Ag level more markedly ($p=0.010$, Exp: 0.136, 95%CI: 0.030-0.621) than anti-viral drug treatment ($p=0.164$, Exp: 1.793,

95%CI: 0.788-4.079) according to Cox proportional hazards regression analysis. Four patients in the delayed ES gargling group received anti-viral drugs from admission onwards, but the duration of the Ag-positive period was not shortened in these cases. The effects of the anti-viral drugs on the durations of the Ag-positive period and hospitalization period were not evaluable in this study model (Figures 3C and D). A randomized controlled study is required to evaluate these effects of anti-viral drug treatments.

Among the examined clinical factors, being free of symptoms was found to be associated with a shorter Ag-positive period in

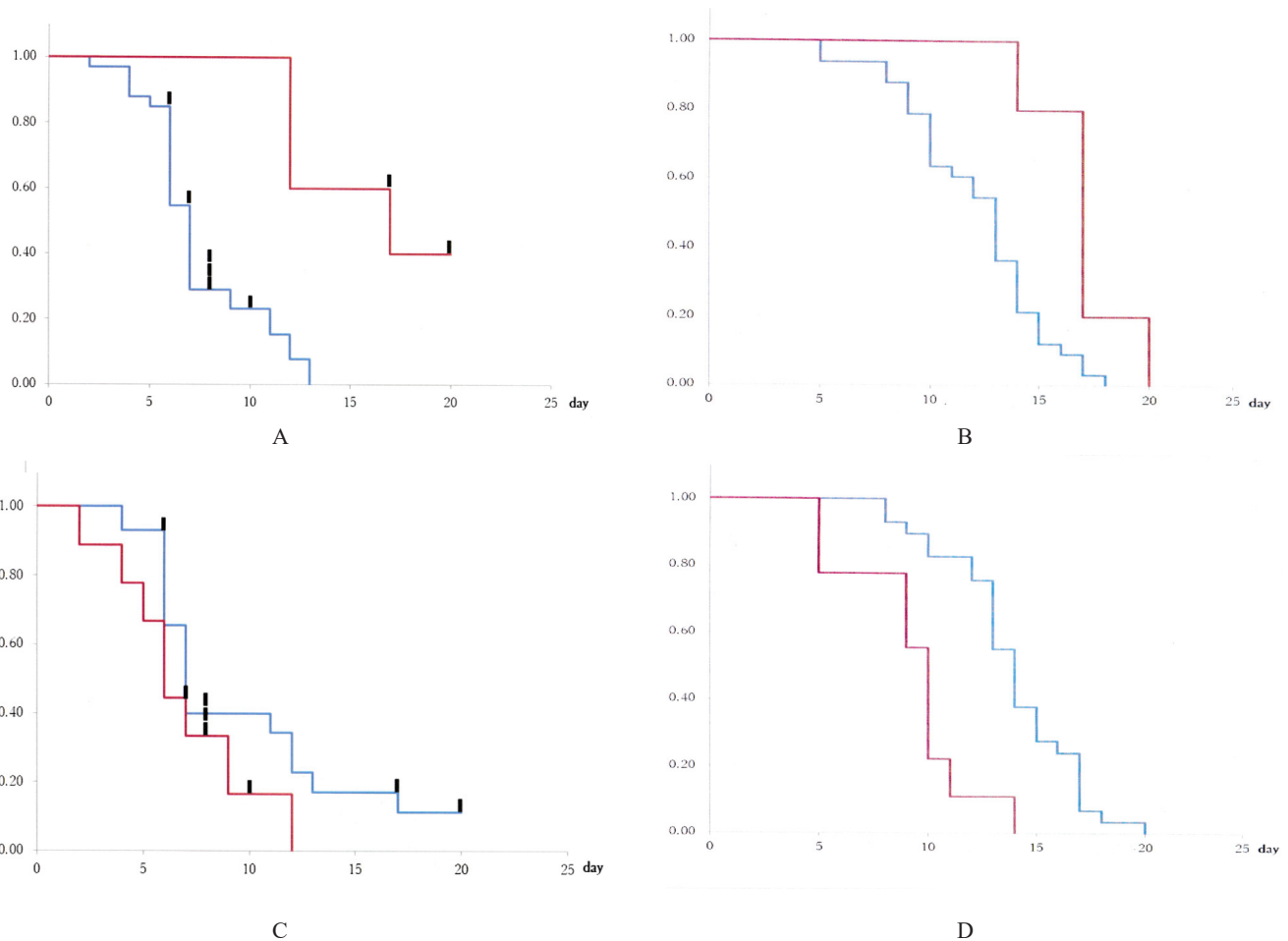


Figure 3: Salivary SARS-CoV-2 antigen positivity rate and hospitalization rate.

Salivary SARS-CoV-2 antigen (Ag) positivity rate: Initial electrolyzed saline (ES) gargling vs. delayed ES gargling.

Time to an Ag positivity rate of 50%: study group ($n=33$): 7 days, control group ($n=5$): 17 days (log-rank test: $p=0.0013$); Cox proportional hazards ratio: 0.136, 95%CI: 0.030-0.621, $p=0.010$.

Blue line: Initial gargling (study) group; red line: delayed gargling (control) group; black columns: discharged cases with Ag levels of <20 pg/ml.

(B) Hospitalization rate: Initial ES group vs. delayed ES group.

Time to a hospitalization rate of 50%: study group: 13 days, control group: 17 days (log-rank test: $p=0.0041$).

(C) Salivary Ag positivity rate after admission: Anti-viral drug treatment group vs. no anti-viral drug treatment group.

Time to an Ag positivity rate of 50%: anti-viral drug treatment group ($n=29$): 7 days, no anti-viral drug treatment group ($n=9$): 6 days (log-rank test: $p=0.0785$); Cox proportional hazards ratio: 1.793, 95%CI: 0.788-4.079, $p=0.164$.

Blue line: Anti-viral drug treatment group; red line: no anti-viral drug treatment group; black columns: discharged cases with Ag levels of <20 pg/ml.

(D) Hospitalization rate: Anti-viral drug treatment group vs. no anti-viral drug group.

Time to a hospitalization rate of 50%: Anti-viral drug group: 14 days, no anti-viral drug group: 10 days (log-rank test: $p<0.001$).

the Cox proportional hazards regression analysis ($p=0.0476$, Exp: 0.377, 95%CI: 0.144-0.990). On the other hand, exhibiting pneumonia shadows, the initial Ag level, and vaccination status were not found to be related to the duration of the Ag-positive period. In COVID-19, the presence/absence of pneumonia lesions at an early stage may not be affected by the viral infection itself, but by cytokine reactions. In addition, the pharyngeal mucosa may be the main site at which the SARS-CoV-2 virus proliferates, and it may be possible to instantly decontaminate large amounts of superficial virus at an early stage by gargling ES. On the other hand, proliferating parasite virus particles may be decontaminated as they leave host cells by gargling ES daily. It should be noted that the patients in the study group who had received all of their scheduled vaccinations might not have been fully immunized against COVID-19 (Table 3).

Table 3: Influence of clinical features on the effects of treatment strategies on the duration of the salivary antigen-positive period (Cox proportional hazards model).

Parameters	No. of patients	P-value	Exp	95%CI
Pneumonia shadows: no/yes	14/24	0.0999	0.5257	0.2444-1.1309
Initial antigen level: <500 pg / ≥500 pg	13/25	0.0895	0.4976	0.2224-1.1136
Initial symptoms: no/yes	5/33	0.0476	0.3772	0.1437-0.9899
Fully vaccinated: yes/no	13/25	0.6598	0.8341	0.3720-1.8704

Exp: hazard ratio, CI: confidence interval.

The preventative effects of blowing up a balloon against the progression of pneumonia were not evaluable in this study, nor were the adverse effects of excessive oxygenation, as only one patient with pneumonia required oxygenation for dyspnea.

Due to the prophylactic use of ES (ES is sprayed on the face and into the nasal cavity), none of our hospital staff have been infected with COVID-19 since the beginning of the pandemic.

Discussion

Viral pneumonia is rare among patients with conventional community-acquired pneumonia [6]. However, COVID-19 is associated with a high incidence of comorbid pneumonia [14], which can be alarming for patients, and the treatment procedures currently employed for COVID-19 in Japan result in a mortality rate of 1~2% [13]. COVID-19 can rapidly progress to pneumonia and can result in respiratory failure (14). Two thirds of our patients already had pneumonia on admission. The in-hospital mortality rate of COVID-19 has been reported to be as high as 23.2% in some studies [14].

There are no first aid procedures for novel infectious biohazards, except quarantine. We have routinely used ES as a disinfectant and sterilizer against the bio contamination of residential spaces, medical equipment, skin, mucosae, and the closed pleural/

abdominal cavity since 1993 [1,4]. In addition, we routinely use ES gargling for preoperative oral hygiene to prevent bio contamination during tracheal intubation. We have also applied ES gargling as a first aid disinfectant against COVID-19 bio contamination. In a preliminary study, we reported that ES instantly disinfected COVID-19 [5].

The effects of anti-viral drugs were not examined in this study. However, the early stage use of anti-viral drugs may be effective against COVID-19, as it reduced the duration of the Ag-positive period in more severe cases to a similar duration to that seen in asymptomatic cases. On the other hand, local disinfection through gargling ES blocked the progression of the disease. Specifically, daily ES gargling reduced the numbers of superficial and parasitic virus particles within/on host cells within a week. None of our patients with pneumonia progressed to severe states that required mechanical ventilation for respiratory failure, and all of their pneumonia shadows faded away as the salivary Ag level reduced [5]. Our treatment strategy achieved a 50% Ag-positive rate within 7 days and a 50% hospitalization rate within 13 days. In addition, all of the patients were discharged from hospital without their pneumonia progressing within 20 days; thus, there were no cases of in-hospital mortality. These results are superior to those of the current treatment strategies for COVID-19 [14].

The reason why local control suppresses systemic disease, e.g., pleural drainage for septic empyema promptly suppresses general symptoms in the absence of systemic antibiotics [1], is unclear. The following hypotheses regarding COVID-19 are suggested: 1. SARS-CoV-2 has the ability to rapidly proliferate at the primary infection site. 2. The primary site is the main source of virus particles and/or chemical mediators. 3. The mechanism responsible for rapid disease progression involves the hematological dissemination of the virus and/or chemical mediators, rather than dissemination through airways, which have multiple defense mechanisms against viruses [15], from an early phase. 4. The lungs are the first receiver of venous or lymphatic return from the affected site. The clinical features of COVID-19 resemble those of adult respiratory distress syndrome [10], and the main target organ is the pulmonary vascular endothelium [16]. Alveolar capillaries with thin walls in the basement membrane and endothelium are involved in gas exchange in the low-pressure circulatory system [11,17]. These capillaries are sensitive to cytokine reactions [17]. The multiple simultaneous non-segmental pulmonary lesions and ground glass opacities seen in the early phase of the infection are due to interstitial permeability edema [15,18]. 5. Residual virus particles in pulmonary interstitial spaces are removed by anti-viral drugs, macrophages, or other factors in the pulmonary lobules [15,18]. Based on the abovementioned hypotheses, we developed the following treatment strategy against COVID-19: 1. immediate local decontamination with ES irrigation reduces the levels of the virus and cytokine producers and blocks the hematological spread of the virus and cytokine reactions. However, our data suggested that the virus might not infect endothelial cells in the early phase of pneumonia, as pneumonia shadows faded as the salivary Ag level

was reduced by local control of the virus through ES gargling, and only minimal organic changes were seen [5]. Tissue Ag test through transbronchial lung biopsy of pneumonia lesion is required to discriminate between viral pneumonitis and interstitial edema produced by cytokine reactions. However, it is difficult to exclude Ag contamination from upper and large airways during tissue sampling. 2. Appropriate anti-viral drugs should be administered [8,9]; however, the effects of such drugs were not evaluable in this study. Anti-viral drugs may be effective, as they were used in more severe cases in this study, and the duration of the Ag-positive period in this group was almost the same as that seen in the no anti-viral drug treatment group, who had no symptoms. 3. Inducing PEEP [10] through a simple procedure (blowing up a balloon or a rubber glove) might be helpful for suppressing pulmonary edema in the reversible phase, but this was not evaluable in this study. Excess amounts of oxygen can damage interstitial tissue [10,11], but the toxicity of oxygenation was also not examined in this study.

Fumigation with biomass smoke is a traditional procedure used against the bio contamination of indoor communal spaces in Buddhist countries, including Japan [4]. Biomass smoke shows disinfection activity against airborne and droplet-borne bio contamination [4]. However, indoor smoking has been regulated in Japan since April 1, 2020, which coincides with the beginning of the first COVID-19 pandemic wave in our country [13]. Hypochlorite is the only remaining reserved disinfectant for viral bio contamination [3].

Among the various sources of hypochlorite, ES is a non-toxic all-purpose disinfectant, which kills viruses, bacteria, and fungi [1-3]. ES is a novel disinfectant, which was developed in Japan 1987, and has demonstrated disinfection activity against 25 viruses [3]. The US FDA approved ES as a high-level disinfectant in October 2002 [3]. ES is a low-dose hypochlorite-containing disinfectant (Cl⁻ content: swimming pool water: 1 ppm, ES: 20~60 ppm, bleach: 5,000~60,000 ppm) [2,3]. It has no adverse effects on the human body or the environment because it is immediately converted to water after coming into contact with an organic substance or being exposed to the air [3]. The antimicrobial mechanism of ES is based on the effects of hypochlorite and superoxide [2] under a super acidic state (pH 2.2–2.8). A super acidic state conveys hydro lipid affinity on ES solution [4]. Due to its lipid affinity, ES instantly binds to and passes through viral envelopes and bacterial cell walls. Viruses may be more susceptible to ES than bacteria because they lack cell walls and do not form biofilms. In a previous study, gargling a glass of ES almost completely eradicated oral bacteria and fungi [4]. ES can sterilize bacteria and fungi within a minute, and in practical terms 10 seconds is sufficient to achieve sterilization [1]. Current pharyngeal antiseptics do not exhibit virucidal activity against COVID-19 [19]. Among current disinfectants, ES solution is the only one that can be used for virucidal decontamination of the human body [3]. Therefore, ES can be used as a first aid disinfectant against viral bio contamination of skin and mucosae.

The process used to produce ES solution is quite simple. Also,

the associated production costs are quite low; i.e., it costs approximately 1 US dollar to produce 1000 liters of ES [20]. In addition, ES generators are widely used for hospital sanitation and for rinsing fresh vegetables, seafood, and flowers in Japan; therefore, the immediate mass-volume production of ES solution is feasible [4]. However, the long-term routine use of ES gargling dissolves dental enamel; thus, nasal use may be better than oral use. In addition, ES can disturb blood coagulation during active hemorrhaging, although pressing a finger on the affected site is often sufficient to control such bleeding. ES has a foul smell, as it contains hypochlorite, but this is an indicator of its disinfection activity. ES also discolors some dyes and metal plates, and rusts some metals [4].

Our study provides clinical evidence of the virucidal activity of ES, but an in vitro study, e.g., involving viral cultures, is required to definitively confirm the virucidal activity of ES against SARS-COV-2, as current Ag test covers both live and dead virus. Transbronchial lung biopsy is required to support our hypothesis. ES could be a first aid key solution for novel biohazards.

Conclusion

ES can be used as a first aid disinfectant against the progression and droplet-borne transmission of COVID-19.

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