Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Acidic electrolyzed water potently inactivates SARS-CoV-2 depending on the amount of free available chlorine contacting with the virus

Yohei Takeda a, Hiroshi Uchiumi b, Sachiko Matsuda c, Haruko Ogawa c, * 

a Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada, Obihiro, Hokkaido, 080-8555, Japan 
b ACT Corporation, 16 Chome 2-2, Odori, Obihiro, Hokkaido, 080-0010, Japan 
c Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada, Obihiro, Hokkaido, 080-8555, Japan

ABSTRACT

Alcohol-based disinfectant shortage is a serious concern in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Acidic electrolyzed water (EW) with a high concentration of free available chlorine (FAC) shows strong antimicrobial activity against bacteria, fungi, and viruses. Here, we assessed the SARS-CoV-2-inactivating efficacy of acidic EW for use as an alternative disinfectant. The quick virucidal effect of acidic EW depended on the concentrations of contained-FAC. The effect completely disappeared in acidic EW in which FAC was lost owing to long-time storage after generation. In addition, the virucidal activity increased proportionately with the volume of acidic EW mixed with the virus solution when the FAC concentration in EW was same. These findings suggest that the virucidal activity of acidic EW against SARS-CoV-2 depends on the amount of FAC contacting the virus.

1. Introduction

Patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were first reported in China in December 2019 [1]. On June 22, 2020, the World Health Organization (WHO) reported >8.8 million confirmed SARS-CoV-2 cases and >460,000 deaths worldwide [2]. It is fundamentally important to decrease human-to-human infection by enforcing strict, practically sustainable control measures such as providing a sufficient amount of antiviral disinfectant to people. However, the worldwide demand has led to a shortage of alcohol-based disinfectants that effectively inactivate SARS-CoV-2 [3].

Acidic electrolyzed water (EW) with a high concentration of free available chlorine (FAC) shows strong antimicrobial activity against bacteria, fungi, and viruses. EW does not harm humans compared to chlorine, so its application to agricultural and food industries is approved [4–6]. An EW generator simultaneously produces a large amount of hypochlorous acid EW and strong alkaline water by electrolyzing water containing NaCl or KCl in an electrolysis chamber. Acidic EW has a virucidal effect against human immunodeficiency virus, hepatitis B virus, herpes simplex viruses, norovirus, influenza A virus, and food-and-mouth disease virus [7–12]. This study evaluated the virucidal effect of EW against SARS-CoV-2 in order to facilitate its wide usage as an alternative disinfectant and contribute to SARS-CoV-2 control.

2. Materials and methods

2.1. Test solutions

The pH 2.5 and FAC concentrations (66–109 ppm) of NaCl-free EW (Clean Refre; Act Co., Obihiro, Japan) was generated using a three-compartment Clean Fine electrolyzer (Act Co.). The difference in FAC concentrations was due to the difference in the production lot of EW. Each EW sample was stored in a tightly capped shade bottle at room temperature and used within 9 days. In addition, acidic EW samples (pH 2.7) with a low FAC concentrations (23 and 2 ppm) were prepared by leaving the fresh acidic EW for 17 and 31 days after generation, respectively, without closing the shade bottle cap. Double-distilled water (DDW) was used as a control.
2.2. Virus and cells

SARS-CoV-2 (JPN/TY/WK-521 strain) and VeroE6/TMPRSS2 cells [13] were obtained from the National Institute of Infectious Diseases (Tokyo, Japan). VeroE6/TMPRSS2 cells were inoculated with SARS-CoV-2 and then cultured in virus growth medium containing Dulbecco’s modified Eagle’s medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 1% fetal bovine serum (FBS), 20 mM l-glutamine (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 100 μg/mL of kanamycin (Meiji Seika Pharma Co., Ltd., Tokyo, Japan).

![Fig. 1. Evaluation of SARS-CoV-2-inactivating activity of acidic EW. (A) 1% FBS-containing SARS-CoV-2 solution was mixed with DDW and acidic EW (pH 2.5, FAC 74 ppm) at virus:test solution ratios of 1:1, 1:5, and 1:9. The reaction time was 1 min. (B) 1%–40% FBS-containing SARS-CoV-2 solution was mixed with DDW and acidic EW (pH 2.5, FAC 74 ppm) at a 1:9 virus:test solution ratio. The reaction time was 1 min. (C) 1% and 40% FBS-containing SARS-CoV-2 solution was mixed with DDW and acidic EW (pH 2.5, FAC 66 ppm) at virus:test solution ratio of 1:20. The reaction time was 1 min. (A–C) Error bars indicate mean ± SD (n = 4 per group). Student’s t-test was performed to analyze statistical significance between the DDW and acidic EW groups; **P < 0.01; ***P < 0.001.

![Fig. 2. Comparison of the SARS-CoV-2-inactivating activity of acidic EWs with different FAC concentrations. (A) 1% FBS-containing SARS-CoV-2 solution was mixed with DDW, fresh (day 0) EW (pH 2.5, FAC 109 ppm) and 17 day-stored EW (pH 2.7, FAC 23 ppm) at a 1:9 virus:test solution ratio. The reaction time was 1 min. (B) 1% FBS-containing SARS-CoV-2 solution was mixed with DDW; fresh (day 0) EW (pH 2.5, FAC 105 ppm) and 31 day-stored EW (pH 2.7, FAC 2 ppm) at a 1:9 virus:test solution ratio. The reaction time was 1 min. (A, B) Error bars indicate mean ± SD (n = 4 per group). The Kruskal–Wallis test with Dunn’s multiple comparison test was performed to analyze statistical significance among all the groups; *P < 0.05; **P < 0.01.]

Y. Takeda et al. / Biochemical and Biophysical Research Communications 530 (2020) 1–3
2.4. Statistical analysis

Student’s t-test was performed to analyze statistically significant differences between the two groups. The Kruskal–Wallis test with Dunn’s multiple comparison test was performed to analyze statistical significance among the three groups. P values < 0.05 were used to determine statistical significance.

3. Results and discussion

First, the SARS-CoV-2-inactivating activities of acidic EWs (pH 2.5, FAC 74 ppm) with different virus:acidic EW ratios were evaluated. We mixed 1% FBS-containing SARS-CoV-2 solution with DDW or acidic EW in virus:test solution ratios of 1:1, 1:5, and 1:9. After a 1-min reaction, the acidic EW potently inactivated SARS-CoV-2 using 9 times volume of SARS-CoV-2 solution, and the viral titer of acidic EW-treated SARS-CoV-2 solution was below the detection limit (≥99.99% inactivation; decrease of >4.25 log10 TCID50/mL). However, its activity decreased when using 5 times volume and was unrecognizable when using an equal volume (Fig. 1A). Next, to evaluate the effect of protein in the virus solution on the virucidal activity of acidic EW, 1% FBS concentration (Fig. 1B). On the other hand, at a 1:20 virus:test solution ratio, acidic EW (pH 2.5, FAC 66 ppm) potently inactivated the 40% FBS-containing SARS-CoV-2 solution (below the detection limit) (Fig. 1C).

Next, we compared the virucidal activities of fresh (day 0) EW with DDW or acidic EW in virus:test solution ratios ranging from 1:1 to 1:20. Virus-containing mixtures were placed for 1 min at 25 °C and then inoculated into cells, and a tenfold serial dilution was performed. After incubation for 3 days, a cytopathic effect was observed, and TCID50/mL was calculated using the Behrens–Kärber method [14].

Declaration of competing interest

The authors declared no conflict of interest.

Acknowledgments

We would like to thank Enago (https://www.enago.jp) for English language editing. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhao, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (2020) 727–733, https://doi.org/10.1056/NEJMoa2001017.
[2] World Health Organization, reportCoronavirus disease (COVID-2019) situa
tion reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports (accessed 23 June 2020).
[3] A. Kretzel, D. Todt, P. Vlokoval, S. Stein*er, M. Gultom, T.T.N. Thao, N. Ebert, M. Holwerda, J. Steinmann, D. Niemeyer, R. Dijkman, G. Kampf, C. Drosten, E. Stennmann, V. Thiel, S. Pflander, Inactivation of severe acute respiratory syndrome coronavirus 2 by WHO-recommended hand rub formulations and alcohols, Emerg. Infect. Dis. 26 (2020), https://doi.org/10.3201/ eid2607.200915.
[4] Y.R. Huang, Y.C. Hung, S.Y. Hu, Y.W. Huang, D.F. Hwang, Application of electrolyzed water in the food industry, Food Contr. 19 (2008) 329–345, https://doi.org/10.1016/j.foodcont.2007.08.012.
[5] R.M.S. Thorn, S.W.H. Lee, G.M. Robinson, J. Greenman, D.M. Reynolds, Electrochemically activated solutions: evidence for antimicrobial efficacy and applications in healthcare environments, Eur. J. Clin. Microbiol. Infect. Dis. 31 (2012) 641–653, https://doi.org/10.1007/s10096-011-1369-9.
[6] Y. Chen, H. Xie, J. Tang, M. Lin, Y.C. Hung, H. Lin, Effects of acidified electrolyzed water treatment on storability, quality attributes and nutritive properties of longan fruit during storage, Food Chem. 320 (2020) 126641, https://doi.org/10.1016/j.foodchem.2020.126641.
[7] C. Morita, K. Sano, S. Morimatsu, H. Kiura, T. Goto, T. Kohno, W. Hong, H. Miyoshi, A. Iwasawa, Y. Nakamura, M. Tagawa, O. Yokosuka, H. Saisho, T. Tsuji, Y. Katsutaka, Disinfection potential of electrolyzed solutions containing sodium chloride at low concentrations, J. Virol Methods 85 (2000) 163–174, https://doi.org/10.1016/S0166-0934(00)00165-2.
[8] W.P. Geun, D.M. Boston, J.A. Kase, M.N. Sampson, M.D. Sobsey, Evaluation of liquid- and fog-based application of sterilx hypochlorous acid solution for surface inactivation of human norovirus, Appl. Environ. Microbiol. 73 (2007) 4463–4468, https://doi.org/10.1128/AEM.02839-06.
[9] M. Tagawa, Inactivation of a hepadnavirus by electrolyzed acid water, J. Antimicrob. Chemother. 46 (2000) 363–368, https://doi.org/10.1093/jac/46.3.363.
[10] N. Tanaka, T. Fujisawa, T. Daimon, K. Fujisawa, N. Tanaka, M. Yamamoto, T. Abe, The effect of electrolyzed strong acid aqueous solution on hemodial
ysis equipment, Artif. Organs 23 (1999) 1055–1062, https://doi.org/10.1111/j.1525-1594.1999.tb2244x.
[11] S. Tamaki, V.N. Bui, L.H. Ngo, H. Ogawa, K. Imai, Virucidal effect of acidic electrolyzed water and neutral electrolyzed water on avian influenza viruses, Arch. Virol. 159 (2014) 405–412, https://doi.org/10.1007/s00705-013-1840-2.
[12] V.N. Bui, K.V. Nguyen, N.T. Pham, A.N. Bui, T.D. Dao, T.T. Nguyen, H.T. Nguyen, D.Q. Trinh, K. Inui, H. Uchiyuni, H. Ogawa, K. Imai, Potential of electrolyzed water for disinfection of foot-and-mouth disease virus, J. Vet. Med. Sci. 79 (2017) 726–729, https://doi.org/10.1292/jvms.16-0614.
[13] N. Nao, K. Sato, J. Yamagishi, M. Tahara, Y. Nakatsu, F. Seki, H. Katoh, A. Ohtsuka, Y. Shirogane, M. Hayashi, T. Suzuki, H. Kikuta, H. Nishimura, M. Takeda, Consensus and variations in cell line specificity among human metapneumovirus strains, PLoS One 14 (2019), https://doi.org/10.1371/jour
dl.pone.0215822.e0215822.
[14] G. Kärber, Beitrag zur kollektiven Behandlung pharmakologischer Rei
enversuche, Naunyn-Schmiedebergs Arch. Pharmacol. 162 (1931) 480–483.