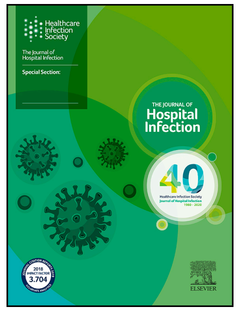

Inactivation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by gaseous ozone treatment

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Infection with severe acute respiratory coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has become a worldwide pandemic [1]. The symptoms of COVID-19 vary widely from asymptomatic disease to pneumonia, and COVID-19 is capable of causing life-threatening complications such as acute respiratory distress syndrome, multisystem organ failure, and ultimately death. Older patients and those with preexisting respiratory or cardio-vascular conditions appear to be at the greatest risk for severe complications.

Ozone gas is effective against the majority of microorganisms tested by numerous research groups, and relatively low concentrations of ozone and short contact time are sufficient to inactivate bacteria, fungus, parasites, and viruses [2–5]. Because of this, ozone should be considered for adoption as an effective weapon in the global fight against COVID-19. In this study, we evaluated the efficacy of ozone gas for inactivation of SARS-CoV-2.

We used the SARS-CoV-2 (JPN/TY/WK-521) strain, which was isolated and provided by the National Institute of Infectious Diseases, Japan. The SARS-CoV-2 culture was performed using VeroE6/TMPRSS2 cells (JCRB1819). Virus culture broths were harvested by two cycles of freezing and thawing and clarified by centrifugation at 10 000 g for 15 min at 4 °C. We subjected the supernatant to ultrafiltration (Amicon Ultra-15; Merck Millipore Ltd., IRL), followed by three washing steps with PBS. A sample (50 µL; 8.5×10^5 PFU) of viral suspension was deposited on a 3 cm^2 area of stainless steel plates. The plates were allowed to dry before exposure to ozone gas and were exposed to ozone immediately after drying. The plates were placed in an ozone-proof airtight acrylic box (H: 23 cm, D: 30 cm, W: 40 cm) with the device generating ozone gas (TM-04OZ; Tamura TECO Ltd., Japan) and were 15 cm away from the device. The plates were then exposed at a concentration of 1.0 ppm ozone for 60 minutes (CT value 60) and 6.0 ppm of ozone at 55 minutes (CT value 330) at temperature 25°C and relative humidity of 60-80%. In each experiment, plates placed for 60 or 55 minutes without ozone exposure were used as controls. Each plate was placed in a 50-ml tube containing 5 mL D-MEM (FUJIFILM Wako Pure Chemical Corporation, Japan), and the solution containing a plate was mixed for 1 minute on a vortex mixer to dislodge any attached virus. The virus titre of SARS-CoV-2 was determined by using the plaque technique on confluent layers of VeroE6/TMPRSS2 cell cultures grown in 12-well culture plates as described previously [6]. This study was conducted in a BSL-3 laboratory at Nara Medical University.

The plaque assay before exposure of ozone was 1.7×10^7 PFU/mL. The titre after exposure of 1.0 ppm ozone at 60 minutes was 1.7×10^4 compared with 5.8×10^5 PFU/mL for the control. After exposure to 6.0 ppm ozone at 55 minutes the titre was less than or equal to 1.0×10^3 PFU/mL, compared with 2.0×10^6 PFU/mL for the control. The titre decreased significantly following exposure to ozone, suggesting that ozone inactivated SARS-CoV-2.

Studies of disinfection with surrogate viruses used ozone concentrations in the range of 10 ppm to 20 ppm for shorter periods [2, 3]. Using higher ozone concentrations for shorter periods may make the process more logistically feasible in busy hospitals where short room turnaround times are required. Although, since it is acknowledged that high concentrations can damage equipment and items, the use of lower concentrations may be desirable in some situations. The low concentration device we used would be better suited for use at night when there are no patients.

The transmission routes of SARS-CoV-2 include droplet transmission, including cough, sneeze, and droplet inhalation transmission. In addition, SARS-CoV-2 may spread by contact transmission and be acquired in numerous indoor public spaces, including hospitals. The surface environment in patient’s room may be frequently contaminated [7, 8], and contact with these contaminated surfaces may result in hand contamination of healthcare personnel that may be transferred to patients. Therefore, there is a need to develop methods of disinfection. Ozone gas can reach every corner of the environment, including sites that might prove difficult to gain access to with conventional liquids and manual cleaning procedures. In addition, ozone gas is very easy to manufacture as it is produced by electrolysis and does not require replenishment of raw materials.

Recently, Blanchard et al. reported ozone disinfected influenza A virus and respiratory syncytial virus that would serve as a reasonable surrogate for SARS-CoV-2 [2]. These results suggested that ozone has an effect on SARS-CoV-2, as we have demonstrated in this study. To our knowledge, this is the first report about the inactivation of SARS-CoV-2 by ozone, and our findings suggest that ozone could be added to the disinfection options for use in the future.

Conflict of interest statement

None declared.

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None.

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