

Virucidal Effect of Gaseous Chlorine Dioxide on Murine Coronavirus A59



Jumi Kim¹, Bo-Hye Shin¹, Kyoung Ju Song², Jong Rak Kim², Kyongmin Kim¹

¹Department of Microbiology, Ajou University School of Medicine



ABSTRACT

Chlorine dioxide, ClO₂, gas is a powerful disinfectant which is 2.5 and 500,000 times more effective than chlorine-based disinfectants and alcohol, respectively. In this study, we examined whether gaseous chlorine dioxide can inactivate murine coronavirus A59. Chlorine dioxide gas was induced from the is a ready-to-use product, without necessitating ClO₂ gas generator on site. The treated concentration of ClO₂ was 0.16ppmv/min. We exposed chlorine dioxide gas directly to the aliquots of murine coronavirus A59 to examine the virucidal effect of chlorine dioxide gas. To begin with, we activated chlorine dioxide batch for 11 hrs, then aliquots of viruses were exposed to chlorine dioxide gas for 1, 6, or 12 hrs, respectively and titrated by plaque assays on delayed brain tumor cells. After 6 hrs, the titer of gaseous chlorine dioxide-exposed virus was about 3.5 times lower than that of non-exposed control virus. Compared to 9.5 x 10³ plaque forming unit/ml of murine coronavirus A59 after 12 hrs of non-exposure, there were no viable virus after 12 hrs exposure. It should be noted here that gaseous chlorine dioxide can inactivate murine coronavirus A59 in 8% normal bovine serum containing viral inoculum, demonstrating that chlorine dioxide gas may act as a virucide even in high concentrations of organic material. Taken together, the gaseous chlorine dioxide may suggest the new paradigm of disinfection system to block the pathogenic viral infections from abroad and the secondary infections, therefore preventing the drastic socio-economic impact by emerging viral infections, which we have learned from MERS outbreak.

MATERIALS and METHODS

(A) Virucidal Effect of Chlorine Dioxide gas on Influenza A virus

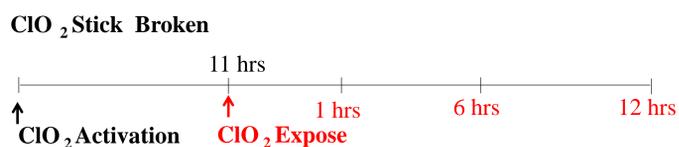
1. Activate chlorine dioxide batch for 11 hrs before exposure to viruses (Broke)
2. Aliquots of H1N1 Influenza A virus in 24 well plate were exposed to chlorine dioxide gas for 1, 6, and 12 hrs.
3. Titrate Influenza A virus by plaque assay on MDCK cells.

(B) Virucidal Effect of Chlorine Dioxide gas on murine coronavirus A59

1. Activate chlorine dioxide batch for 11 hrs before exposure to viruses (Broke).
2. Aliquots of murine coronavirus A59 in 24 well plate were exposed to chlorine dioxide gas for 1, 6, and 12 hrs.
3. Titrate murine coronavirus A59 by plaque assay on DBT cells.

RESULTS

(A) Virucidal effect of Influenza A virus



	(Titer)	
	Control	3.7 x 10 ⁵
ClO ₂ (-)	1 hrs	2.4 x 10 ⁵
	6 hrs	1.1 x 10 ⁵
	12 hrs	5.1 x 10 ⁴
ClO ₂ (+)	1 hrs	2.4 x 10 ⁵
	6 hrs	1.3 x 10 ⁴
	12 hrs	2.7 x 10 ²

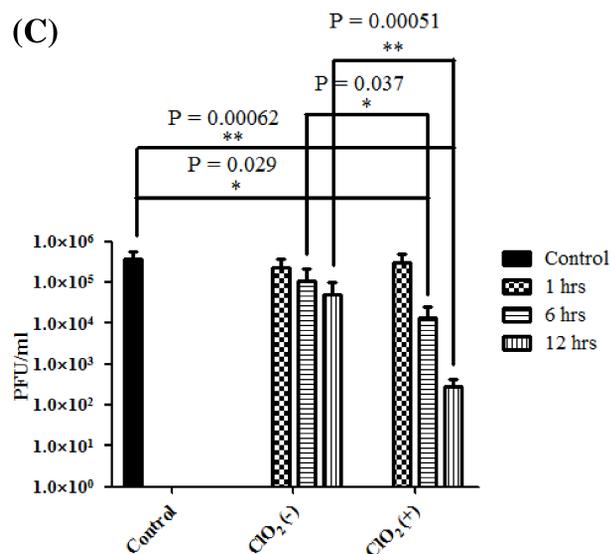
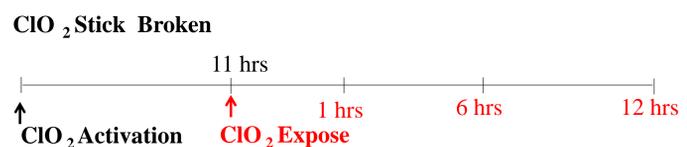


Fig 1. Virucidal effect of chlorine dioxide on Influenza A viruses. Aliquots of Influenza A viruses were directly exposed to the chlorine dioxide gas. We activated chlorine dioxide batches for 11 hrs, then place each batch to the sealed box with aliquot of viruses in it. Aliquoted viruses will be exposed to chlorine dioxide gas for 1, 6, or 12 hrs, respectively, and titrated by plaque assay on MDCK cells. Titer of control virus, H1N1 3rd, was 3.7 x 10⁵ PFU/ml. Compared to non-exposure control virus, virus titers were decreased about 8.3 times after exposure for 6 hrs. Also, Virus titers were reduced about 186 times after 12 hrs exposure. 4 independent experiments were performed and results are shown as mean ± SD (*p<0.05, **p<0.01).

(A) Virucidal effect of murine coronavirus A59



	(Titer)	
	Control	3.1 x 10 ⁶
ClO ₂ (-)	1 hrs	1.0 x 10 ⁶
	6 hrs	1.4 x 10 ⁵
	12 hrs	9.5 x 10 ³
ClO ₂ (+)	1 hrs	1.0 x 10 ⁶
	6 hrs	4.0 x 10 ⁴
	12 hrs	0

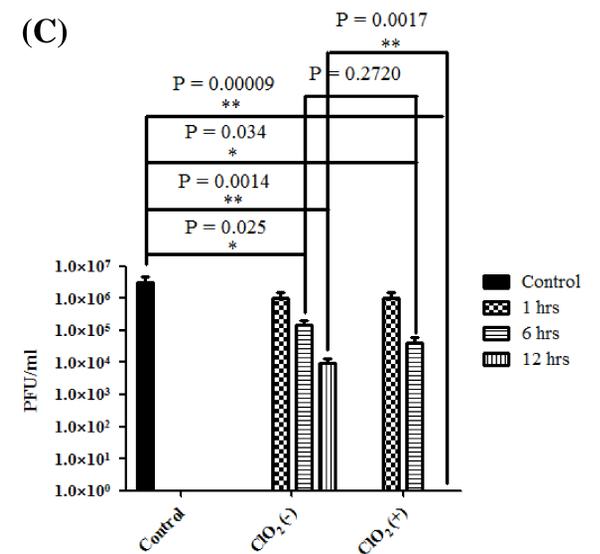


Fig 2. Virucidal effect of chlorine dioxide on murine coronavirus A59. Murine coronavirus A59 were exposed to the chlorine dioxide gas for 1, 6, or 12 hrs, respectively, and then we determined the titer of viruses by plaque assay on DBT cells. Control virus titer was 3.1 x 10⁶ PFU/ml. Virus titers were decreased about 3.5 times after exposure for 6 hrs. There were no viable virus after 12 hrs exposure. 4 independent experiments were performed and results are shown as mean ± SD (*p<0.05, **p<0.01).

CONCLUSION

- After 6 hrs of ClO₂ exposure, the titers of ClO₂-exposed Influenza A virus and murine coronavirus A59 were decreased about 8.3 and 3.5 times, respectively, than those of non-exposed control viruses.
- Also after 12 hrs exposure, Influenza A virus titer was 186 times lower than the control.
- After 12 hrs exposure, there were no viable murine coronavirus A59 virus after 12 hrs exposure.
- Taken together, the gaseous chlorine dioxide may suggest the new paradigm of disinfection system to block the pathogenic viral infections.