Usefulness of Chlorine Dioxide to Airborne Bacteria at a Hospital Using Biological Information

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생물학적 정보를 활용한 병원에서 존재하는 공기중 부유 세균에 대한 이산화염소의 유용성

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요 약 본 연구에서, 세균의 생물학적 정보와 이산화염소 가스의 생화학적 정보들을 활용하여, 그람 양성 세균인 *Alloiococcus otitis, Erysipelothrix rhusiopathiae, Staphylococcus caprae, Staphylococcus lentus* 및 그람 음성 세균인 *Acinetobacter baumannii complex, Aeromonas salmonicida, Brucella melitensis, Oligella ureolytica*에 대한 이산화염소가스의 성장 억제 효과를 분석하였다. 전체적으로, 이산화염소 가스는 10 CFU 미만으로 세균의 성장을 약 99 % 억제하였다. 하지만, 그람 양성인 *Alloiococcus otitis* 및 그람 음성인 *Aeromonas salmonicida*는 약 50 CFU 이상인 것으로 밝혀졌다. 여러 세균과의 실험 결과를 비교할 때, 이산화염소 가스의 농도는 세균 억제에 대해 10 ppm 내지 400 ppm 일 것이라고 제안한다. 이 연구의 결과는 이산화염소 가스의 임상적 유용성을 평가하기 위한 기본 데이터로 사용될 수 있을 것이다. 이 연구가 임상에 있는 근무자가 병원에서 감염을 일으키는 미생물의 존재를 인식하고 예방하는 사전 지식에 도움이 되는 경우, 융합분야 중, 임상에서처럼 환자 치료와 같은 활동에 도움이 될 것이다. 향후에, 이산화염소 가스에 대해 억제되는 미생물들의 정보의 데이터를 활용하여, 환자에게 감염된 미생물들을 신속히 억제하는데 기초가되는 연구결과가 될 것으로 사료된다.

주제어 : 세균, 이산화염소, 생물학적 정보, 생화학적 정보, 데이터

Abstract In the present study, using biological information of bacteria and biochemical information of chlorine dioxide gas, Gram-positive bacteria, e.g., *Alloiococcus otitis, Erysipelothrix rhusiopathiae, Staphylococcus caprae, Staphylococcus lentus,* and gram-negative bacteria, e.g., *Acinetobacter baumannii* complex*, Aeromonas salmonicida, Brucella melitensis, Oligella ureolytica* were used whether a plastic kit to release ClO_2 gas could inhibit their growth. Overall, chlorine dioxide gas showed about 99% inhibition of bacterial growth, with less than 10 CFU. However, it was found that Gram positive *Alloiococcus otitis* and Gram negative *Aeromonas salmonicida* had more than about 50 CFU. When comparing the results of experiments with several bacteria, it suggested that the concentration of chlorine dioxide gas would be at least 10 ppm to 400 ppm for the bacterial inhibition. The results of this study could be used as basic data to evaluate the clinical usefulness of chlorine dioxide gas. If this study helps with prior knowledge to help clinicians to recognize and prevent the presence of micro-organisms that cause infections in hospitals, it would be helpful for activities such as patient care as a convergence field. In the future, it is considered that the research results will be the basis for rapidly inhibiting the microbes infected with patients by utilizing data of the information of the microbes that are inhibited for chlorine dioxide gas.

Key Words : Bacteria, Chlorine dioxide, Biological information, Biochemical information, Data

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1. Introduction

Chlorine dioxide, ClO_2 is water-soluble yellow gas of a strong oxidative activity [1, 2], and it is thought to have inhibitory effects to bacteria, yeast, molds and viruses [1, 3-6]. ClO_2 gas is an effective disinfectant agent of a broad spectrum with strong oxidization ability [7]. ClO_2 gas is effective at preventing aerosol-induced influenza virus infection in mice by denaturing viral envelope proteins and [8]. On the other hand, the ClO_2 gas had minimum inhibitory concentration (MIC) to *Bacillus subtilis, Lactobacillus plantarum, Lactobacillus fermentum* and *Leuconostoc mesenteroides* with 10 ppm, 125 ppm, 75 ppm and 50 ppm, respectively [9]. Numbers of viable airborne bacteria in the operating room in a hospital in the presence or absence of 0.03 ppm ClO_2 gas were found to be 10.9 ± 6.7 and 66.8 ± 31.2 colony-forming units (CFU), showing that the 0.03 ppm ClO_2 gas would be effective to inhibit the airborne bacteria [10]. The Chlorine dioxide gas has been applied a lot in crops, with the aim of preventing the growth of bacteria and preventing other infections [11, 12]. In various ways, the application of chlorine dioxide gas has been made. In our previous study, the growth of air-borne *Micrococcus luteus, Granulicatella adiacens*, etc isolated from a hospital were inhibited by ClO_2 gas kindly provided by Purgofarm company. In the present study, Gram-positive bacteria, e.g., *Alloiococcus otitis, Erysipelothrix rhusiopathiae, Staphylococcus caprae, Staphylococcus lentus,* and gram-negative bacteria, e.g., *Acinetobacter baumannii* complex, *Aeromonas salmonicida, Brucella melitensis, Oligella ureolytica* were used whether a plastic kit to release ClO_2 gas could inhibit their growth. Briefly, *A. otitis*, recently named as *A. otitidis*, is associated with middle ear effusions of children with otitis media with effusion (OME) [13, 14, 15]. *E. rhusiopathiae* causes Erysipeloid, BakerRosenbach disease of bacterial skin infection [16]. *S. caprae* is a commensal microorganism observed in joint and bone infections in humans [17]. *S. lentus* is oxidase-positive, coagulase-negative bacterium [18]. *A. baumannii* complex is an opportunistic pathogen in humans and affects immuno-compromised patients as a hospital-derived pathogen [19]. *A. salmonicida* is an infectious agent to fish disease such as salmonids [20]. *B. melitensis* is one of the zoonotic microorganisms and causes 500,000 human cases of brucellosis in the world per year [21]. *O. ureolytica* is observed at the urinary and respiratory tract as a commensal microorganism [22]. In the present study, using biological information of bacteria and biochemical information of chlorine dioxide gas, Gram-positive and -negative bacteria, were used whether a plastic kit to release ClO_2 gas could inhibit their growth.

2. Materials and Methods

2.1 Bacterial culture

The eight bacteria used were previously captured in hospital air and stored at a deep freezer for later use. The bacteria were grown in tryptic soy agar (TSA, MB cell, Korea) enrichment medium, and their colonies were confirmed after 24 hr. The cultured bacteria were observed by Gram staining procedure [27], and biochemical analysis was conducted by VITEK2 (Biomerieux, USA). To obtain the correct CFU, a single colony was diluted with 0.85% NaCl and adjusted to a McFaland turbidity of 0.5 so that about 1.5×10^5 colonies could be grown [27].

2.2 Bacterial growth by ClO_2 gas

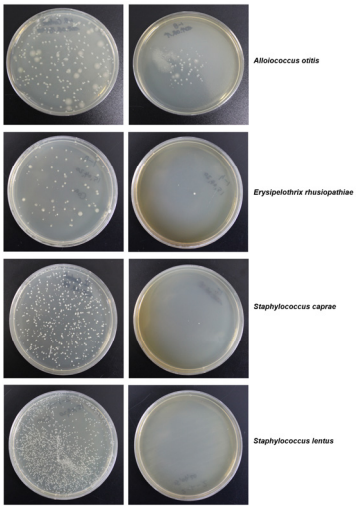
At first, bacteria of 0.5 of McFaland turbidity was mixed with 0.85% NaCl and was cultured onto TSA plates. Cultured bacteria were treated with a plastic stick, farme-Tok, kindly provided by Purgofarm, co, Ltd. (Hwasung, Gyeonggido,

Korea) to release ClO_2 gas. In order to confirm the growth of bacteria and to check the effectiveness and suppress the leakage of chlorine dioxide gas, a chamber (250W×350D×200H) was used. After 24 hours, colonies of bacteria were counted and counted, and the growth inhibitory effect of chlorine dioxide gas was verified.

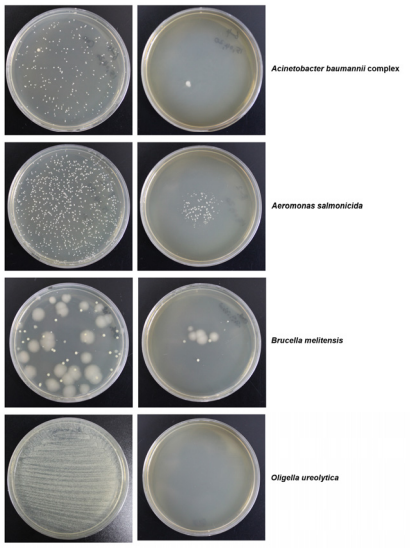
3. Results

3.1 Bacterial identification

Although the selection medium of bacteria is all set separately, in general, TSA medium was used to grow bacteria. Bacterial growth was observed after incubation in TSA medium for 24 hr (Fig. 1 & 2). Based on the biochemical results from VITEK2, accurate identification of bacteria and gram staining was performed once again. Four gram positive- and negative-bacteria were applied to observe the effect of ClO_2 gas.



[Fig. 1] Gram-positive bacterial growth by a plastic stick releasing ClO_2 gas



[Fig. 2] Gram-negative bacterial growth by a plastic stick releasing ClO_2 gas

3.2 Analysis of bacterial growth by ClO_2 gas

To confirm the effect of chlorine dioxide gas, the colonies of the bacteria were observed after 24 hours with the colonies where the bacteria grew. The plastic stick, Farme-Tok, produced the carbon dioxide gas mentioned above. In order to prevent the leakage of chlorine dioxide gas, bacteria were cultured simultaneously with a plastic kit in the plastic chamber. Bacteria were completely streaked in TSA medium, and CFU was calculated after incubation for 24 hr (Table 1). Overall, chlorine dioxide gas showed about 99% inhibition of bacterial growth, with less than 10 CFU. However, it was found that Gram positive *A. otitis* and Gram positive *B. melitensis* and *A. salmonicida* had more than about 50 CFU (Fig. 1 & 2). Colonies of uninhibited bacteria remained in the middle of the TSA medium, and chlorine dioxide gas was found to inhibit bacteria present from the edge of the TSA plate. Taken together, these results suggested that chlorine dioxide gas with biochemical utility ccould provide data or a database to effectively inhibit bacteria by utilizing biological information of used bacteria.

〈Table 1〉 Counting of bacterial CFU by a plastic stick releasing ClO_2 gas at O h and 24 hr.

Gram staining	Bacteria	Treatment of ClO_2	*CFU/㎖ at O hr incubation **	CFU/㎖ after 24 hr incubation	Decreasing percentage
positive	*Alloiococcus otitis*	-	1.5×10^4	-	-
		ClO_2	1.5×10^4	< 50	99.9
	Erysipelothrix rhusiopathiae	-	1.5×10^5	-	-
		ClO_2	1.5×10^5	< 10	99.9
	Staphylococcus caprae	-	1.5×10^3	-	-
		ClO_2	1.5×10^3	< 10	99.9
	Staphylococcus lentus	-	1.5×10^4	-	-
		ClO_2	1.5×10^4	< 10	99.9
negative	*Acinetobacter baumannii complex*	-	1.5×10^4	-	-
		ClO_2	1.5×10^4	< 10	99.9
	Aeromonas salmonicida	-	1.5×10^5		
		ClO_2	1.5×10^5	< 10	99.9
	Brucella melitensis	-	1.5×10^4	-	-
		ClO_2	1.5×10^4	< 50	99.9
	Oligella ureolytica	-	1.5×10^5	-	-
		ClO_2	1.5×10^5	< 10	99.9

* CFU : Colony Forming Unit
** CFU/㎖ was adjusted by 0.5 of McFarland index.

4. Discussion

All microorganisms of *A. otitis, E. rhusiopathiae, S. caprae, S. lentus, A. baumannii* complex, *A. salmonicida, B. melitensis, O. ureolytica* applied in this study are not known in several references to be essential for infection in hospitals. However, they have been collected and cultured from the air in hospitals in Pyeongtaek, South Korea and could be associated with hospital-acquired infection [23, 24, 25, 26]. This study aimed to observe the effect of a plastic stick releasing chlorine dioxide gas on clinical bacteria present in the air in a hospital and to evaluate its potential for future clinical application. In the present study, using biological information of bacteria and biochemical information of chlorine dioxide gas, Gram-positive and -negative bacteria, were used whether a plastic kit to release ClO_2 gas could inhibit their growth. In this study, all bacteria were inhibited 99% for growth by the ClO_2 gas. As considered with initial CFU, only under 10 bacterial colonies were grown except for *A. otitis* and *B. melitensis* of about 50 colonies. The plastic stick of "FarmeTok" released ClO_2 (13 ppmv/hr) gas and was sufficient to inhibit the bacterial growth. The plastic stick of "FarmeTok" releases ClO_2 gas by 13 ppmv per hr [27] and completely inhibited the bacteria above. In fact, the plastic stick emitted 312 ppm in 24 hours because chlorine dioxide gas emitted 13 ppm per hour. Lowe et al (2003) reported that the 385 ppm concentration of chlorine dioxide was sufficient to inhibit bacterial growth such as *Acinetobacter baumannii, Escherichia coli, Enterococcus faecalis* and *Mycobacterium smegmatis* exhibited at a hospital [28]. As mentioned above, the ClO_2 gas concentration varied 10 ppm to 125 ppm to inhibit the growth of *Bacillus subtilis, Lactobacillus plantarum, Lactobacillus fermentum* and *Leuconostoc mesenteroides* [9]. When comparing the results of experiments with several bacteria and the results in this study, it suggested that the concentration of chlorine dioxide gas would be at least 10 ppm to 400 ppm to sufficiently inhibit bacterial growth for 24 hr. However, noteworthy is also the change in the concentration of chlorine dioxide gas due to differences in the pathogenicity of the bacteria in future. The inhibition mechanism of ClO_2 is unclear, but the action of ClO_2 is only known as inhibition. For porcine reproductive and respiratory syndrome virus (PRRSV), ClO_2 could inhibit the first stage of viral life, which inhibited binding itself to cells where PRRSV was not internalized and released [29]. It is not well known about the inhibition mechanism of ClO_2. On the other hand, ClO_2 was able to inhibit the binding of PRRSV to non-internalized cells by inhibiting the first stage of viral life for porcine genital and respiratory syndrome virus (PRRSV) [29]. The results of this study could be used as basic data to evaluate the clinical usefulness of chlorine dioxide gas. However, it is expected that the exact mechanism of reacting with the bacteria of chlorine dioxide gas will be verified by cell

biology and molecular biology in near future. This study can be applied to several fields such as the convergence field. If this study helps with prior knowledge to help clinicians to recognize and prevent the presence of micro-organisms that cause infections in hospitals, it would be helpful for activities such as patient care [30]. In the future, it is considered that the research results will be the basis for rapidly inhibiting the microbes infected with patients by utilizing data of the information of the microbes that are inhibited for chlorine dioxide gas.

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