수의학석사학위논문

차아염소산수의 병원성 미생물에 대한 항균 효과

(Antimicrobial effect of hypochlorous acid on pathogenic microorganisms)

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이 논문을 수의학석사학위논문으로 제출함

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Abstract

Antimicrobial effect of hypochlorous acid on pathogenic microorganisms

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Public health has been defined as the science and art of preventing disease, prolonging life and promoting health through the organized efforts and informed choices of society, organizations, public and private, communities and individuals . Environmental health(or environmental hygiene) is a branch of public health concerned with all aspects of the natural and built environment that may affect human health. Other phrases that concern or refer to the discipline of environmental health include environmental public health and environmental health and protection. Disinfectants in terms of public health safety have been used

to destory microorganism on non-living objects and sanitizer is a agent to decrease a level of number of microorganisms present on the surface of the abiotic material. Hypochlorous acid (HOCl) is a weak acid and dissociates to the hypochlorite ion (-OCl) and proton (H+) depending on the solution pH. Both HOCl and the -OCl are strong oxidizing agents and show germicidal activity. Previous studies have reported that HOCl is 80 times more effective to *Escherichia coli* and 40 times more effective to *Pseudmnas* spp. than -OCl. We evaluated the germicidal activity of HOCL against various pathogenic microbes. Thirty-one ATCC strains were exposed to HOCL solution at various concentrations (20, 40 and 80 ppm) for 1 minute. All the strains of bacteria, yeasts and mycobacteria were killed at 80 ppm after exposure to HOCL. The results suggest that HOCL solution could be used to effectively disinfect public areas.

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keywords : disinfectant, public health, hypochlorous acid, pathogenic microbes

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I. Introduction

Public health has been defined as the science and art of preventing disease, prolonging life and promoting health through the organized efforts and informed choices of society, organizations, public and private, communities and individuals [14]. Environmental health(or environmental hygiene) is a branch of public health concerned with all aspects of the natural and built environment that may affect human health. Other phrases that concern or refer to the discipline of environmental health include environmental public health and environmental health and protection. Disinfectants in terms of public health safety have been used to destory microorganism on non-living objects and sanitizer is a agent to decrease a level of number of microorganisms present on the surface of the abiotic material [13]. Hypochlorous acid (HOCl) is a weak acid and dissociates to the hypochlorite ion (-OCl) and proton (H+) depending on the solution pH. Both HOCl and the -OCl are strong oxidizing agents and show germicidal activity [9]. Previous studies have reported that HOCl is 80 times more effective to *Escherichia coli* and 40 times more effective to *Pseudmnas* spp. than - OCl [10, 12]. However, until now HOCl has simply been believed as a transient by-product in the ubiquitous chlorine disinfectants because of its difficulty of isolation and purification. We made high concentration and stable HOCl by the patent

technology (patent Number 10-1051312). The purpose of this study, therefore, was to evaluate the disinfectant activity of HOCl against various pathogenic microbes. Our results indicated that HOCl is an effective disinfectant.

II. Materials & Methods

HOCL solution (3,000 ppm) was diluted with phosphate buffered saline (PBS) to 20, 40 and 80 ppm. Two *Staphylococcus aureus* subsp. *aureus*, 2 *Enterococcus faecalis*, 2 *Escherichia coli* 2 *Pseudomonas aeruginosa*, 3 Methicillin resistant *Staphylococcus aureus* (MRSA), *Salmonella enterica* subsp. *enterica* (*typhimurium*), *Shigella sonnei, Shigella flexneri , Bacillus thuringiensis*, *Bacillus cereus*, *Bacillus cereus, Bacillus circulans*, *Paenibacillus alvei* were cultured in Tryptic Soy Broth (TSB) at 37℃ (*Bacillus* sp. and *Paenibacillus alvei* at 30℃) for 24 h. 8 *Candida albicans* were cultured in Yeast extract-Malt extract Broth (YMB) at 37℃ for 24 h. *Mycobacterium chelonae* subsp. *chelonae*, *Mycobacterium fortuitum* subsp. *fortuitum, Mycobacterium smegmatis* were cultured 3% OGAWA media at 37℃ for 24 h. *Listonella anguillarum* was cultured in 1% TSB at 25℃ for 24 h. All strains diluted with PBS to over 10^8CFU/ml and its 0.1 ml was mixed to 9.9 ml HOCl solution. After 1 min 0.1ml mixtures were inoculated on Tryptic soy agar (TSA), Yeast extract-Malt extract Agar (YMA), 3% OGAWA media and 1% NaCl TSA and incubated at optimal temperature described above for 24 - 48 h.

III. Results

Almost strains were killed at 40 ppm especially *Mycobacterium* spp. and *L. anguillarum* were inactivated at 20 ppm while *Bacillus* spp. and *P.alvei* were not recovered at 80 ppm. The results of this study were provided in Table 1. Previous study have been reported that HOCL showed germicidal effect within 0.5- 1 min at 80 - 100 ppm [5, 6]. Our result indicatedthat HOCL has good bactericidal effect on *Mycobacterium* spp. over 20 ppm and it is similar to previous reported result [11]. Also, *S. aureus* subsp. *aureus*, *E. faecalis*, *E. coli, P.aeruginosa*, MRSA, *S. typhimurium*, *S. sonnei, S. flexneri* and *C. albicans* were inactivated at 40 ppm. However, *Bacillus* sp. and *Panibacillus alvei* were killed at 80 ppm. Previous studies [5, 6] mainly explained the germicidal effect on pathogenic microbes at 80 - 100 ppm HOCL. However, we explained germicidal effects at lower concentration and more pathogenics. In addition, HOCL used in this study can be made as neutral pH and a higher concentration than the previous product [5, 6].

HOCL is 80 times more effective as a sanitizing agent than an equivalent concentration of the OCL- [10, 12]. HOCL, themost effective form of chlorine compounds, kills microbial cells. Molecules that have highly nucleophilic sites are supposed to react rapidly with HOCl. Among the cellular components, these include porphyrins and hemes,

ferredoxin-like iron-sulfur centers, purine and pyrimidine bases, conjugated polyenes, amines, amino acids, and sulfhydryl groups [1]. The oxidation of these components by HOCl results in the loss of physiological functions. During HOCl stress for *E coli*, loss of catalytic function of sulfhydryl enzymes and decrease in antioxidants such as glutathione have been suggested to be the bactericidal events [1, 7]. HOCl has also been found to disrupt oxidative phosphorylation [3], metabolic pathways involved inATP utilization or generation [2], and other membrane-associated activities [4]. Furthermore, HOCl can cause DNA damage resulting from the formation of chlorinated derivatives of nucleotide bases [8].

Table 1. Antimicrobial effect of hypochlorous acid according to the concentration

Bacterial species (ATCC[1] No)	Initial concentration	20ppm	40ppm	80ppm
Bacillus cereus (11778)	3.26×10^6	NE[2]	1.21×10^3	<10
Bacillus cereus (21366)	4.18×10^6	NE	NE	<10
Bacillus circulans (9500)	2.72×10^6	NE	NE	NDC[3]
Bacillus thuringiensis (10792)	5.17×10^6	NE	3.57×10^3	<10
Candida albicans (10259)	4.26×10^6	7.8 x10	NDC	NDC
Candida albicans (10261)	7.12×10^6	4.68×10^2	NDC	NDC
Candida albicans (10231)	1.21×10^6	NDC	NDC	NDC
Candida albicans (18804)	3.97×10^6	5.12×10^2	NDC	NDC

Candida albicans (11006)	1.77×10^6	NDC	NDC	NDC
Candida albicans (18814)	3.24×10^6	4.1 x 10	NDC	NDC
Candida albicans (22972)	5.11×10^6	2.45×10^2	NDC	NDC
Candida albicans (28471)	5.32×10^6	3.11×10^2	NDC	NDC
Enterococcus faecalis (29212)	1.74×10^6	NE	NDC	NDC
Enterococcus faecalis (19433)	4.25×10^6	NE	NDC	NDC
Escherichia coli (25922)	6.11×10^6	NE	NDC	NDC
Escherichia coli (11105)	3.43×10^6	NE	NDC	NDC
Listonella anguillarum (19264)	2.82×10^6	NDC	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (33591)	5.39×10^6	NE	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (33593)	3.06×10^6	NE	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (6538)	2.52×10^6	NE	NDC	NDC
Mycobacterium chelonae subsp. *chelonae* (35752)	1.39×10^6	NDC	NDC	NDC
Mycobacterium fortuitum subsp. *fortuitum* (6841)	2.11×10^6	NDC	NDC	NDC
Mycobacterium smegmatis (19420)	2.26×10^6	NDC	NDC	NDC
Paenibacillus alvei (6344)	4.15×10^6	NE	NE	NDC
Pseudomonas aeruginosa (27853)	2.02×10^6	NE	NDC	NDC
Pseudomonas aeruginosa (25619)	3.55×10^6	NE	NDC	NDC
Salmonella enterica subsp. *enterica (typhimurium)* (29629)	5.76×10^6	NE	NDC	NDC
Shigella flexneri (29903)	3.42×10^6	NE	NDC	NDC
Shigella sonnei (25931)	5.79×10^6	NE	NDC	NDC
Staphylococcus aureus subsp. *aureus* (29213)	8.15×10^6	NE	NDC	NDC
Staphylococcus aureus subsp. *aureus* (25923)	5.68×10^6	NE	NDC	NDC

1) ATCC : american type culture collection.
2) NE : no effect.
3) NDC : not detected colony.

IV. Conclusion & Discussion

Based on this study, HOCL solution is recommended as disinfectant for environmental hygiene and it can control infection of pathogenic microbes using 80 ppm at 1 min. It is suitable for use in public places such as hospital, school, station and public bathroom. Before using it, organic material will be removed by cleaning.

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국문초록

차아염소산수의 병원성 미생물에 대한 항균 효과

소독제는 공중 보건 또는 환경 위생의 측면에서 중요하다. 차아염소산수액(HOCl)은 한국에서 소독제로 개발되었다. 우리는 다양한 병원성 미생물에의 HOCl의 살균 활성을 평가 하였다. 31개의 ATCC 균주를 1 분 동안 다양한 농도 (20, 40, 80 PPM)에서의 HOCl 용액에 노출시켰으며 모든 박테리아, 효모 및 마이코 박테리아의 균주의 HOCl에 노출 된 후 80 ppm으로 살멸하였다. 그 결과로 HOCl 용액을 효율적으로 공용 영역을 소독하는 데 사용될 수 있음을 제안 한다.

주요어 : 소독제, 공중보건, 차아염소산수, 병원성미생물

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