Antimicrobial Effects of Electrolytic Products of Sodium Chloride —Comparative Evaluation with Sodium Hypochlorite Solution and Efficacy in Handwashing—

Shigemi HITOMI1), Shigeyoshi BABA1), Hisako YANO3), Yuji MORISAWA2) and Satoshi KIMURAn2)
Department of Infection Control and Prevention1), Department of Infectious Diseases2), University of Tokyo Hospital
Kanagawa Prefectural College of Nursing and Medical Technology3)
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Abstract

We examined the in vitro bactericidal effects and efficacy on handwashing of water containing electrolytic products of sodium chloride (electrolytic water). The electrolytic water, whose pH and concentration of free residual chlorine were 6.7-6.9 and 20-22 ppm, respectively, showed equal reduction of both Staphylococcus aureus and Escherichia coli to dilution of commercially available sodium hypochlorite containing 60 ppm of free residual chlorine. This bactericidal effect was calculated to be due to hypochlorous acid, based on the pH and the amount of chlorine in solution. Handwashing with the electrolytic water reduced the numbers of S. aureus on hands by 1/10², while running water and 0.2% benzalkonium chloride with 80% ethanol gave a 1/10 and 1/10⁵ reduction, respectively. We conclude that electrolytic water might be applicable for handwashing in place of running water.

Introduction

Chlorine and chlorine compounds have been used for disinfection since the nineteenth century. Hypochlorites have a number of advantages in particular, including a broad antimicrobial spectrum, fast action, no poisonous residuals, and low cost, and therefore have been widely used in this field. Many factors affect the biocidal activity of hypochlorites, perhaps the most important of which is pH because hypochlorous acid (HOCl) dissociates with increased the pH into a proton and a hypochlorite ion (OCl⁻), which is much less microbiocidal. In the practical application of these compounds, it is important to consider immediate attenuation in the presence of organic materials and irritation to skin or respiratory system1,2).

Recently, a number of reports have introduced apparatuses which electrolyze solutions containing a small amount of sodium chloride and evaluated the bactericidal products yielded at the anode3)–16). The products effectively disinfected medical instruments such as forceps or scissors8),

Correspondence to: Shigemi HITOMI
Department of Infection Control and Prevention University of Tokyo Hospital, 7-3-1 Hongo Bunkyo-ku, Tokyo 113-8655, Japan
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gastroendoscopes\textsuperscript{12}, and hemodialysis equipment\textsuperscript{14}. They also worked effectively in handwashing\textsuperscript{9,13,16}. Although their bactericidal effects may largely be attributed to chlorine compounds in solution\textsuperscript{4}, no report has compared the effect quantitatively with that of hypochlorite solution, which may be most widely used compound for disinfection. Therefore, we examined the in vitro bactericidal effect of water containing the electrolytic products of sodium chloride (electrolytic water) quantitatively and compared it with that of sodium hypochlorite (SH) solutions. In addition, we evaluated the efficacy of electrolytic water for handwashing.

Materials and Methods

1. Characteristics of electrolytic water and SH solutions

The electrolytic water which we investigated had a pH of 6.7–6.9 (at 25°C); and a concentration of free residual chlorine (FRC), measured with the N,N-diethyl-p-phenylenediamine (DPD) method\textsuperscript{17}, of 20–22 ppm. the generating apparatus (manufactured by T • R • P Co., Ltd., Osaka) worked for 15 sec and yielded 1.5 l of electrolytic water in one operation. The produced solution flowed automatically from a nozzle of the apparatus.

Various strengths of SH solutions were made by mixing a commercially available hypochlorite solution (Purelox\textsuperscript{®} [Oyalox, Tokyo]) into distilled water. FRC concentrations were also measured with the PPD method. The pH of the SH solution was 8.3–8.4 (at 25°C) at a concentration of 20 ppm.

2. Bactericidal effect of the electrolytic water and diluted SH solutions

\textit{Escherichia coli} (the ATCC 11229 strain) and \textit{Staphylococcus aureus} (a clinical isolate) were cultured overnight in Nutrient Broth (DIFCO Laboratories, Detroit) and 0.5 ml of each culture solution was re-cultured in 4.5 ml of fresh Nutrient Broth for two more hours. The re-cultured bacteria were washed twice and suspended in sterile distilled water. Ninety microliters of the suspensions, containing approximately $4 \times 10^6$ colony forming units (CFU), were mixed with 10 µl of either electrolytic water or SH solutions (containing 20, 40, or 60 ppm of FRC) at 25°C for 30 sec. After the reaction was stopped by adding 900 µl of Nutrient Broth, the numbers of surviving bacteria were counted by culturing on agar plates for two days at 35°C.

3. Antimicrobial effect of the electrolytic water in handwashing

Prior to the test, we explained the objects, procedures, and anticipated side effects of the experiment to volunteers and confirmed that they had no macroscopic cut or scratch and no irritation with 70% ethanol on their hands. Five hundreds microliters of \textit{S. aureus} (a clinical isolate) solution, which had been cultured overnight to approximately $5 \times 10^8$ CFU per ml in Nutrient Broth, were evenly spread on their hands which had been washed beforehand with non-medical soap. After one hand was covered with a plastic glove (control side), the volunteers washed their hands for 15 sec with either: 1. running water; 2. running electrolytic water; or 3. approximately 3 ml of 0.2% benzalkonium chloride with 80% ethanol. After the handwashing and removal of residual liquid with paper towels, the other hand was covered with a plastic glove (handwashed side) and bacteria remaining on both of the hands were collected separately by the following rubbing method: 20 ml of sampling buffer (0.7% Tween-80, 0.1% lecithin, 0.5% sodium thiosulfate) was spread inside the glove, then rubbed on the fingertips for 2 sec, between the fingers for 2 sec, except between the thumb and forefinger which was rubbed for 4 sec, rubbed on the palm for 10 sec, on the dorsum for 10 sec, then rubbing between the fingers and fingertips was repeated, and 500 µl of sampling buffer was collected. The buffer was diluted stepwise with sterile saline and cultured on mannitol-salt agar plates (Eiken Kagaku, Tokyo) at 35°C for 48 hr. The number of \textit{S. aureus} colonies was counted. For final evaluation, we calculated the ratios of the number of recovered bacteria from the handwashed side to that from the control side (CFU ratios).

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Results

1. Comparison of in vitro bactericidal effect between the SH solutions and electrolytic water

We repeated the experiment four times and calculated medians of surviving bacteria for analysis. As shown in Fig. 1, the SH solutions reduced living *E. coli* from $4 \times 10^6$ CFU (range: $3.6 \times 10^6 - 4.1 \times 10^6$ CFU) to $1 \times 10^6$ (9 × 10⁵–4 × 10⁶) CFU at an FRC concentration of 20 ppm, $1 \times 10^8$ (3 × 10⁷–3 × 10⁸) CFU at 40 ppm, and to $5 \times 10^4$ (2 × 10³–8 × 10⁴) CFU at 60 ppm within 30 sec. In the same manner, *S. aureus* decreased from $4 \times 10^6$ (3.3 × 10⁶–4.7 × 10⁶) CFU to $2 \times 10^6$ (2 × 10⁵–3 × 10⁶) CFU at 20 ppm, 1 × 10⁶ (1.0 × 10⁵–1.2 × 10⁶) CFU at 40 ppm, and to $5 \times 10^4$ (3 × 10³–6 × 10⁴) CFU at 60 ppm. Although *E. coli* exhibited more sensitivity to the SH solutions than *S. aureus*, both species showed an exponential decrease in numbers of surviving cells according to the FRC concentration in the SH solutions.

Electrolytic water, tested in exactly the same way, reduced the numbers of living *E. coli* and *S. aureus* from $4 \times 10^6$ CFU to $4 \times 10^4$–8 × 10⁴) CFU and $6 \times 10^5$ (3 × 10⁵–1 × 10⁶) CFU, respectively, equivalent to the SH solution containing 60 ppm of FRC (Fig. 1). Thus, the bactericidal effect of electrolytic water was equivalent to an SH solution with an FRC concentration of 60 ppm.

2. Reduction of bacteria in handwashing

The handwashing test was separately repeated six times. The median recovery rate on the control side, i.e. the ratio of the cells recovered from the sampling buffer to those attached to one hand prior to the test, was 57.0% (range: 6.3–113%). The median CFU ratios of handwashing with running water (HRW), the running electrolytic water (HEW), and 0.2% benzalkonium chloride with water were 76.2%, 97.4%, and 99.9%, respectively.
80% ethanol (HBE) were $2 \times 10^{-1}$ (range: $4 \times 10^{-2}$–$2 \times 10^{-1}$), $1 \times 10^{-2}$ ($5 \times 10^{-3}$–$4 \times 10^{-2}$), and $2 \times 10^{-5}$ ($<10^{-5}$–$1 \times 10^{-3}$), respectively (Fig. 2). There were significant differences between the CFU ratios of HEW and HRW and between HEW and HBE (Mann-Whitney test, $p<0.01$ in both cases). According to these median CFU ratios, handwashing with running electrolytic water reduced the number of bacteria on hands by approximately $1/10^2$, compared to a $1/10$ and $1/10^5$ decrease with running water and 0.2% benzalkonium chloride with 80% ethanol, respectively.

**Discussion**

The germicidal effect of chlorine compounds in water may largely depend on the concentration of HOCl. This molecule theoretically possesses about 80-fold greater bactericidal potency as OCI$^-$$^{18}$. HOCl, which equilibrates with OCI$^-$ in accordance with pH, accounts for 23.3% of chlorine compounds at a pH of 8.0, and 75.2% at neutral pH$^{18}$. Therefore, a hypochlorite solution of pH 7.0 may have equal bactericidal potency to that containing approximately three times as much FRC at pH 8.0. Based on this, one of our findings that electrolytic water had nearly equivalent bactericidal potency to an SH solution containing threefold more FRC, indicates that chlorine compounds account for the full bactericidal effect of electrolytic water and that its higher potency results from a

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**Fig. 2** Comparison of bacterial reductions between three different handwashing methods. Approximately $5 \times 10^8$ CFU of *S. aureus* were attached to volunteers' hands and then one hand was covered with a plastic glove. After handwashing with running tapwater (A), running electrolytic water (B), or 3 ml of 0.2% benzalkonium chloride with 80% ethanol (C), residual bacteria on both hands were collected separately but a rubbing method and cultured on mannitol-salt agar plates. Bacterial reduction was calculated as the CFU ratio of the residual bacteria on the uncovered (handwashed) hand to that on the covered (control) hand (marked as ●). Marks ★ show the medians with each method.
difference in pH.
Neutralization of SH solutions may produce a disinfectant as active as electrolytic water, however, a decrease in pH reduces the stability of chlorine in solution\(^1\). Therefore, SH solutions should be maintained alkaline for longer storage periods. Since electrolytic water can be produced just before use, the instability of chlorine in solution at neutral pH may not be a problem in practical use.

Handwashing is the most important single procedure for reducing the transmission of micro-organisms\(^1\). The transmission of *S. aureus*, which frequently causes severe nosocomial infection, mostly occurs via health care workers whose hands or gloves have been transiently contaminated\(^9\). This is one reason why we used this species in our handwashing tests. The electrolytic water removed 99% of the bacteria on hands whereas running water only removed 90%, indicating a 10-fold greater bactericidal potency for electrolytic water. Since fresh electrolytic water. The findings that 0.2% benzalkonium chloride with 80% ethanol showed markedly greater bacterial reduction than the electrolytic water, indicates that the electrolytic water is less appropriate for hand disinfection than tincture.

During the handwashing tests, we did not observe any adverse reactions such as a skin rash on the volunteers' hands. The electrolytic water, which may work with less consumption of chlorine than the SH solution, is less likely to cause these disadvantages. Sekiya et al. applied another electrolytic water that was more acidic than our electrolytic water to chronic ulcers and reported its effectiveness in conservative treatment\(^7\). Iwasawa et al. showed that another electrolytic water was less toxic to cultured cells than usual disinfectants such as ethanol or povidone-iodine\(^6\). To date, no serious side effects of these newly introduced disinfectants have been reported. However, since the chlorine or its compounds are essentially irritants, close attention must be payed as they are applied to humans.

**References**


食塩電気分解水の抗菌効果
一次亜塩素酸ナトリウム溶液との比較検討および手洗いにおける有効性—

東京大学医学部附属病院感染性制御部1, 感性症内科2)
神奈川県立衛生短期大学3)
人見 重見1) 馬場 重好1) 矢野 久子3)
森沢 雄司2) 木村 哲1,2)

要 旨
食塩電気分解水の抗菌効果と手洗いに使用した場合の有効性について検討した。この電気分解水の pH と遊離残留塩素濃度はそれぞれ6.7-6.9, 20-22ppm だった。黄色プドウ球菌と大腸菌に対する殺菌力を調べたところ、両菌種に対して遊離残留塩素濃度が60ppm の希釈次亜塩素酸溶液とほぼ同等の殺菌力を示した。これらのデータから計算すると、塩素の量と pH 値によって決まる次亜塩素酸の量だけがこの電気分解水の殺菌力を規定すると考えた。この電気分解水で手を洗ったところ、付着させた黄色プドウ球菌を1/10 に減少させた。流水および擦込式エタノールでの減少度はそれぞれ1/10, 1/100 だった。ってこの電気分解水は、手洗いに流水の代わりとして使用することができると結論した。