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# Effect of Electrolyzed Strong Acid Water on Peritoneal Irrigation of Experimental Perforated Peritonitis

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**Abstract**
***Purpose.*** Electrolyzed strong acid water (ESAW) is generated by the electrolysis of a weak sodium chloride solution. Although ESAW is known to have a strong bactericidal activity and to be harmless to the living body, its effectiveness and safety in the treatment of perforated peritonitis has not been well established.
***Methods.*** Male Wistar rats were used for the study. Three hours after cecal ligation and puncture, the cecum was resected and the peritoneal cavity was irrigated with 50 ml of saline (Group S, $n$=12) or ESAW (Group E, $n$=14). The 5-day survival rate was compared between the two groups. In another pair of animals ($n$=10 each), bacteria in the ascitic fluid were counted at 6 and 18 h after irrigation.
***Results.*** No adverse effects of ESAW were observed in the experimental group. The 5-day survival rate was 25% (3/12) and 85.7% (12/14) in Groups S and E, respectively ($P < 0.01$). The bacterial count at 18 h after the irrigation in Groups S and E was $(5.0 \pm 2.5) \times 10^5$/ml and $(2.2 \pm 2.0) \times 10^4$/ml, respectively ($P < 0.0001$).
***Conclusion.*** Peritoneal lavage with ESAW had no adverse effect, and achieved more effective decontamination than saline for perforated peritonitis. Therefore, the results of this study are considered to warrant and support the clinical application of ESAW.

**Key words** Perforated peritonitis · Peritoneal lavage · Electrolyzed strong acid water · Hypochlorous acid water · Bactericidal activity

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## Introduction

Electrolyzed strong acid water (ESAW) is generated by the electrolysis of a weak sodium chloride solution (20 mM NaCl). Its physiological and chemical characteristics include a pH of 2.7, an oxidative-reduction potential of 1000–1100 mV, a dissolved oxygen concentration of 10–30 ppm, and a chlorine concentration of 30–40 ppm, or 1.2 mM as hypochlorous acid,[1,2] which has strong bactericidal activity[2–5] and disinfection potential against viruses.[6–8] The feasibility and safety of ESAW irrigation in wound infection or ocular surface disinfection have been confirmed in animal models.[9,10] However, few applications of body cavity lavage with ESAW in clinical cases have been reported in the literature.[11–14] In order to establish its safety and effectiveness for peritoneal irrigation in perforated peritonitis, an experimental study was conducted.

## Materials and Methods

### *Subjects*

Wistar male rats (Nihon Clea, Tokyo, Japan) 7 weeks of age, weighing about 250 g, which were raised preliminarily on a compound feed (Nihon Aqua, Kyoto, Japan), were used for the study. The animals were randomly divided into two groups (14 rats in each group). Two animals in Group S were lost accidentally.

### *Preparation of Peritonitis*

After fasting for 12 h, the animals were anesthetized with pentobarbital, and after disinfecting the abdomen with povidone iodine, a 2-cm midline laparotomy was performed. Perforated peritonitis was made as described by Wichterman et al.;[15] the cecum was ligated just below the ileocecal valve with 3-0 silk without dissection of the ileocolic vessels, and punctured twice with an 18-gauge needle on the antimesenteric surface. A silicon catheter (inner diameter 0.025 inch, outer diameter 0.045 inch, Dow Corning, Midland, MI, USA) was placed in the superior vena cava via the jugular vein, and physiologic saline was infused at 200 ml/kg per day. The animals were fitted with a harness and swivel so they could move without restraint, and housed in a metabolic cage.

### *Irrigation of the Peritoneal Cavity*

Three hours after cecal ligation and puncture, the animals were prepared using the same procedure, a laparotomy was performed, the cecum was resected, and the peritoneal cavity was irrigated with 50 ml physiologic saline in Group S, and 50 ml ESAW in Group E.

### *Preparation of ESAW*

Electrolyzed strong acid water was generated by the electrolysis of tap water containing 0.12% sodium chloride employing a Model 201 (Nihon Aqua, Kyoto, Japan; Fig. 1). Its chemical characteristics include a pH of 2.7, an oxidative-reduction potential of 1000–1100 mV, a dissolved oxygen concentration of 10–30 ppm, and a chlorine concentration of 30–40 ppm, or 1.2 mM (as hypochlorous acid).

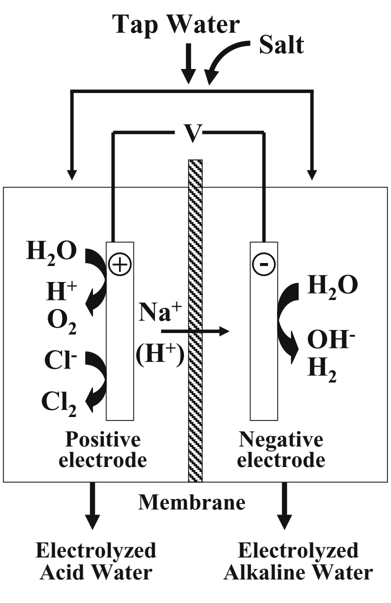


**Fig. 1.** Principle of electrolysis apparatus and formation of electrolyzed strong acid water. When a voltage is applied, water is electrolyzed to give an acid aqueous solution at the positive electrode and a basic aqueous solution at the negative electrode according to the following chemical formulae. On positive electrode: $H_2O \rightarrow 1/2O_2 + 2H^+ + 2e^-$, $2Cl^- \rightarrow Cl_2 + 2e^-$, $Cl_2(aq) + H_2O \leftrightarrow HCl + HClO$; on the negative electrode: $H_2O + 2e^- \rightarrow 1/2H^+ + OH^-$

### *Bacterial Count*

At 6 and 18 h after cecal resection and peritoneal irrigation, half of the animals in both groups were prepared using the same procedure and subjected to a laparotomy. The ascitic fluid was collected from the Douglas pouch. The number of bacteria was counted as colony forming units ($CFU/ml^3$).

### *Statistical Analysis*

The results were expressed as the mean ± SD. A generalized Wilcoxon test was employed for the survival rate, and Student's *t*-test for the bacterial count. Statistical significance was defined as $P < 0.05$.

## Results

### *Survival Rate*

In Group S, 50% of the animals died within 1 day after surgery, and 75% died within 3 days (Fig. 2). The dead animals in Group S became ill with behavior consistent with that described by Wichterman et al. in a sepsis model,[15] and autopsy in Group S revealed mild adhesions in the peritoneal cavity, but no purulent peritonitis, which suggested that the animals died of sepsis. An autopsy in Group E showed no remarkable change in the peritoneal cavity. The survival rate on day 5 in Groups S and E was 25% (3/12) and 85.7% (12/14), respectively, thus being significantly higher in Group E ($P < 0.01$).

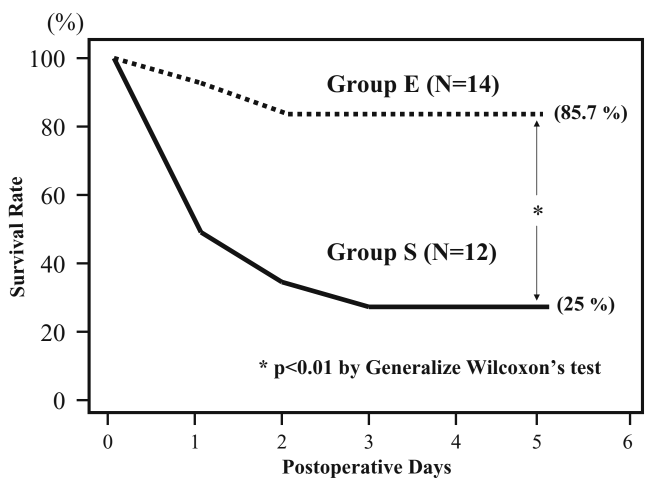


**Fig. 2.** Survival curve. Animals in both groups survived the first day after the surgery. On day 5, the survival rate in Group E (85.7%) was significantly higher than that in Group S (25%) ($P < 0.01$)

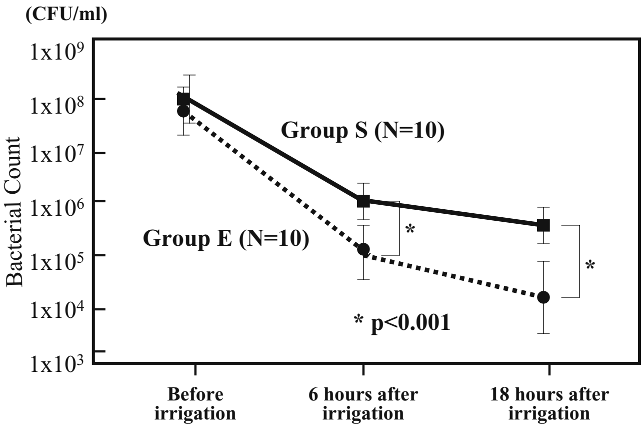


**Fig. 3.** Bacterial count of ascites. The bacterial counts in the ascites both at 6 and 18 h after irrigation were significantly lower in Group E than in Group S ($P < 0.001$)

*Bacterial Count in the Ascitic Fluid*

The bacterial count 6 h after irrigation in Groups S and E was $(3.5 \pm 0.84) \times 10^6$ CFU/ml and $(1.4 \pm 0.35) \times 10^5$ CFU/ml, respectively, while at 18 h after irrigation in Groups S and E it was $(5.0 \pm 2.5) \times 10^5$ CFU/ml and $(2.2 \pm 2.0) \times 10^4$ CFU/ml, respectively (Fig. 3). The bacterial count in the ascitic fluid at both 6 and 18 h after irrigation was significantly lower in Group E than in Group S ($P < 0.001$).

## Discussion

Electrolyzed strong acid water is produced by electrolysis of a weak sodium chloride solution, or tap water with addition of approximately 0.1% NaCl. At the positive electrode, the anode, water ($H_2O$) is transformed to oxygen ($O_2$) and hydrogen ions ($H^+$), chlorite ions ($Cl^-$) to chlorine ($Cl_2$), and then chlorine reacts with water and makes hypochlorous acid (HClO) and hydrochloride (HCl). At the negative electrode, the cathode, water is transformed to hydrogen ($H_2$) and hydroxide ion ($OH^-$; Fig. 1). Electrolyzed strong acid water has the following physiological and chemical properties; a low pH (2.3–2.7), a high oxidative reduction potential (1000–1100 mV), high concentrations of dissolved chlorine (30–40 ppm) and dissolved oxygen (approximately 15–20 ppm), and there is an equilibrium among hypochlorous acid, hypochlorite ions, and chlorine.[1–3,16] Some of these chemical properties, i.e., the low pH (below 3) and high oxidative reduction potential (above 900 mV), do not allow a wide variety of microorganisms to survive.[16] However, Nakagawara et al.[2] demonstrated that hypochlorous acid plays the most important role in the bactericidal activity, and its concentration is quantitatively correlated with the bactericidal activity. Its strong microbicidal activity inhibits the growth of not only a wide spectrum of bacteria including methicillin-resistant *Staphylococcus aureus*,[2–4,17] but also viruses including human immunodeficiency virus and hepatitis B virus;[6–8] moreover, it directly inactivates endotoxin.[18] In addition to its strong microbicidal activity, ESAW has the following advantages over various chemical disinfection agents: it is minimally invasive to tissue because of its extremely low cytotoxicity,[19] it is ecological because its breakdown produces only saline and traces of chloride gas, and last but not least, it is economical because the source is just tap water and salt. Therefore, ESAW has been increasingly used as a disinfectant for medical instruments, such as hemodialysis equipment[18] and endoscopes,[20] and for hand disinfection,[21] and its efficacy and feasibility have also been increasingly recognized in Japan. In addition, ESAW has also been applied for the irrigation of body cavities[11–14] and skin ulcers.[22] From the Japan National Cardiovascular Center, Hayashi et al.[11] reported four cases of postoperative mediastinitis that was successfully treated with ESAW. They reported that ESAW had no adverse effects, while a satisfactory growth of healthy granulation was also observed during convalescence. A controlled study on peritoneal lavage was conducted for perforated peritonitis in pediatric patients.[12] The peritoneal cavity was irrigated with 100 ml/kg normal saline or ESAW, and it was concluded that peritoneal irrigation with ESAW was safe and more effective than irrigation with saline. The peritoneal irrigation with ESAW in experimental peritonitis created by a cecal puncture also proved to be as effective as granulocyte-colony stimulation factor (G-CSF).[23] Gurleyik et al.[23] demonstrated that the subcutaneous injection of 50 mg/kg G-CSF increased the 4-day survival rate from 20% to 90%. Such rates are very close to those observed in the present study, from 25% to 85%. As a result, ESAW is therefore recommended for application in clinical cases; however, only a few prospective studies to establish its safety and effectiveness have so far been reported.[12,14] The current study indicated that peritoneal irrigation of perforated peritonitis with ESAW is a safe and effective method for reducing residual bacteria in the ascitic fluid and could also reduce the incidence of abscess formation in the peritoneal cavity and wound infection, and consequently reduce the length of hospital stay.[12]

## References

1. Kumon K. What is functional water? Artif Organs 1997;21: 2–4.
2. Nakagawara S, Goto T, Nara M, Ozawa Y, Hotta K, Arata Y. Spectroscopic characterization and the pH dependence of bactericidal activity of the aqueous chlorine solution. Analytical Sciences 1998;14:691–8.

3. Hotta K, Kawaguchi K, Saitoh F, Saito N, Suzuki K, Ochi K, et al. Antimicrobial activity of electrolyzed NaCl solutions: effect on the growth of *Streptomyces* spp. Actinomycetol 1994;l8:51–6.
4. Iwasawa A, Nakamura Y. Bactericidal effect of acidic electrolyzed water-comparison of chemical acidic sodium hydrochloride (NaOCl) solution (in Japanese with English abstract). Kansenshogaku Zashi (J Jpn Assoc Infect Dis) 1996;70:915–22.
5. Kiura H, Sano K, Morimatsu S, Nakano T, Morita C, Yamaguchi M, et al. Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. J Microbiol Methods 2002;49: 285–93.
6. Abe S, Miya Y, Okuda R. Inactivation of hepatitis B virus by high oxidation potential water (in Japanese with English abstract). Nihon Shikahozongaku Zasshi (Jpn J Conserv Dent) 1994;37: 1616–23.
7. Morita C, Sano K, Morimatsu S, Kiura H, Goto T, Kohno T, et al. Disinfection potential of electrolyzed solutions containing sodium chloride at low concentrations. J Virol Methods 2000;85: 163–74.
8. Tagawa M, Yamaguchi T, Yokosuka O, Matsutani S, Maeda T, Saisho H. Inactivation of a hepadnavirus by electrolyzed acid water. J Antimicrob Chemother 2000;46:363–8.
9. Nakae H, Inada AH. Effectiveness of electrolyzed oxidized water irrigation in a burn-wound infection model. J Trauma 2000;49: 511–4.
10. Shimomura S, Matsumoto K, Yaguchi H, Okuda T, Miyajima S, Negi A, et al. Acidic electrolysed water in the disinfection of the ocular surface. Exp Eye Res 2000;70:1–6.
11. Hayashi H, Kumon K, Yahagi N, Haruna M, Qatanabe Y, Matsui J, et al. Successful treatment of mediastinitis after cardiovascular surgery using electrolyzed strong acid aqueous solution. Artif Organs 1997;21:39–42.
12. Kubota A, Hoki M, Yonekura T, Nose K, Hirooka S, Kosumi T, et al. Effectiveness of acid oxidative potential water in peritoneal lavage for perforated appendicitis. Asian J Surg 1999;22:282–3.
13. Takemura M, Iwamoto K, Goshi S. intraoperative irrigation with super oxidation water for perforated appendicitis (in Japanese). Geka (Jpn J Surg) 1999;61:1303–5.
14. Ohuchi S, Kawazoe K, Ishihara K, Izumoto H, Eishi K. Management with closed irrigation for post-sternotomy mediastinitis. Jpn J Thorac Cardiovasc Surg 2003;511–4.
15. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock; a review of laboratory models and proposal. J Surg Res 1980; 29:189–201.
16. Hotta K. Acid electrolyzed saline solution: its function and medical application. J Jpn Soc Intensive Care Med 2000;7: 97–105.
17. Iwasawa A, Nakamura Y, Mizuno T. Bactericidal activity of aqua oxidation water against clinically isolated strains (in Japanese). Nihon Kankyo-Kansensyogaku Zasshi (J Jpn Environ Infect) 1993;8:11–6.
18. Tanaka N, Fujisawa T, Daimon T, Fujiwara, Yamamoto M, Abe T. The cleaning and disinfecting of hemodialysis equipment using electrolyzed strong acid aqueous solution. Artif Organs 1999;23: 303–9.
19. Iwasawa A, Nakamura Y. Cytotoxic effect of antiseptics: comparison In vitro, in vivo examination of strong acidic electrolyzed water, povidone-iodine, chlorhexidine and benzalkonium chloride (in Japanese with English abstract). Kansensyogaku Zasshi (J Jpn Assoc Infect Dis) 2003;77:316–22.
20. Sakurai Y, Ogoshi K, Okubo T, Kaku M, Kobayashi I. Strong acidic electrolyzed water: valuable disinfectant of endoscopes. Dig Endoscopy 2002;14:61–6.
21. Otoguro K, Suzuki F, Akimaru Y, Iijima H, Yajima Y, Uebaba K, et al. Hand disinfectant activities of two kinds of electrolysed acid aqueous solutions by globe juice method (in Japanese with English abstract). Nihon Kankyo Kansensyogaku Zasshi (J Jpn Environ Infect) 1996;11:117–22.
22. Sekiya S, Ohmori K, Harii K. Treatment of infectious skin defects or ulcers with electrolyzed strong acid aqueous solution. Artif Organs 1997;21:32–8.
23. Gurleyik G, Yanikkaya G, Gurleyik E, Ozturk E, Dulundu E, Saglam A. Effects of granulocyte-colony stimulation factor on the polymorphonuclear leukocyte activity and the course of sepsis in rats with experimental peritonitis. Surg Today 2007;37:401–5.